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HISTOLOGY OF PLANTS

D. K. Arora
Seema Gupta

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AUTHORS

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Plant Cell

DISCOVERY OF CELLS

Leeuwenhoek ground the best lenses of his time, but many other eager explorers were also using microscopes during the latter half of the seventeenth century. It was a time not unlike our own: an age of intense conflicts and of rapid technical improvements that revolutionized man's view of his environment. Some fifteen years before Leeuwenhoek's first letter to the Royal Society, and Italian, Marcello Malpighi, had seen small blood vessels (later named *capillaries*); and in 1665 Robert Hooke, a secretary of the Royal Society, found interesting microscopic structure in cork (the bark of a Mediterranean oak) and in the stems of various plants. In the cork he observed neat rows of thick-walled compartments that reminded him of a honeycomb. Because of this resemblance he called the compartments "cells." This discovery turned out to be at least as important to biology as was Leeuwenhoek's.

The story of Hooke's "cells" closely parallels the story of Leeuwenhoek's "animalcules." Many men saw both of them during the following century and a half, but no one really understood their significance.

Hooke himself found that in many living materials (the cork, of course, was dead) the cell was filled with a liquid material. Gradually attention shifted to this liquid content. One of the first to call attention to it was a Frenchman of great genius, Jean Baptiste Pierre Antoine de Monel, Chevalier de Eamarck Fig. 1.1.

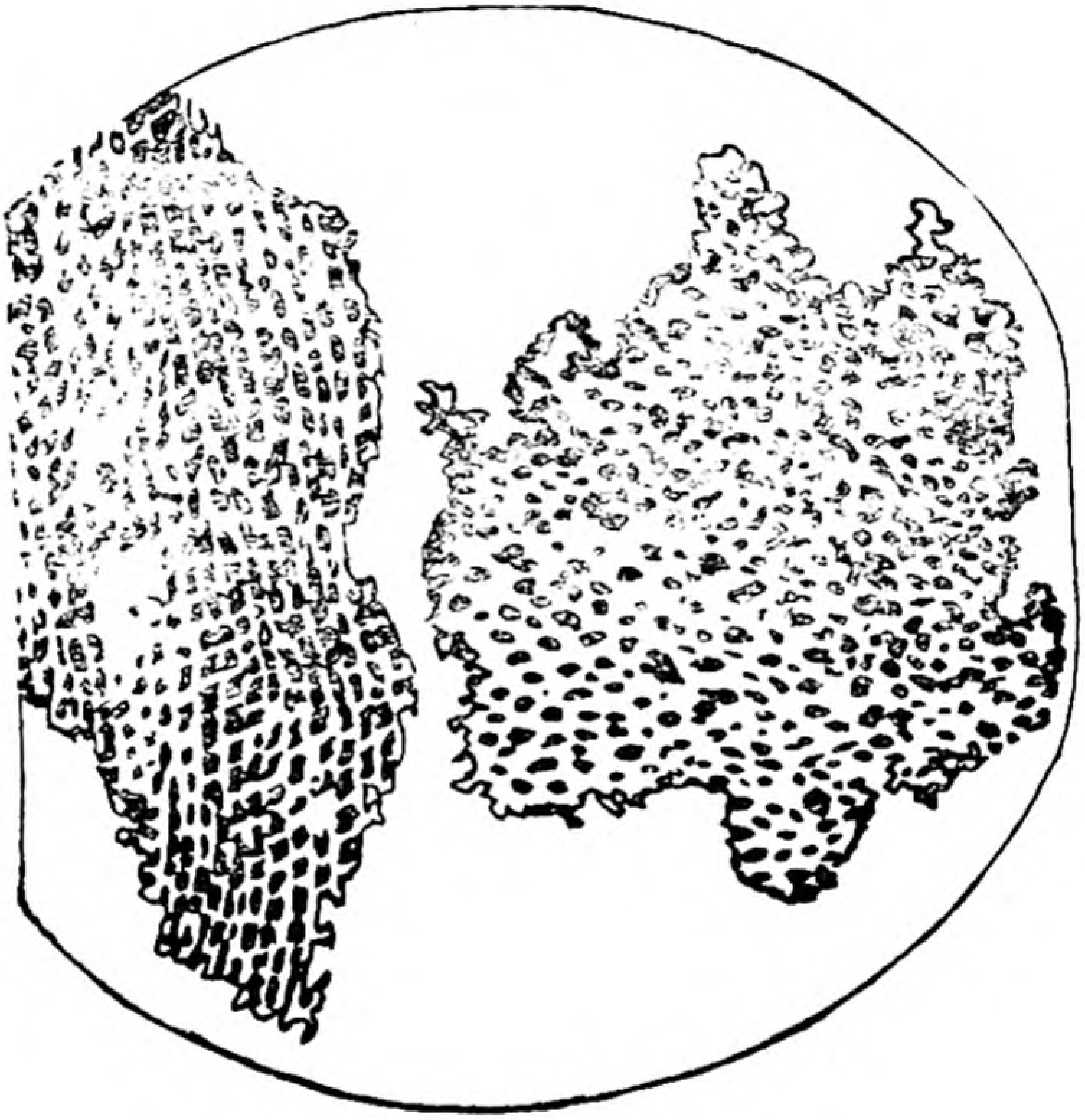


FIG. 1.1: Hooke's drawings of cork, as reproduced in his Micrographia, 1665.

A few investigators were beginning to suspect that all large organisms are constructed of microscopic units of living matter. But there was no science of biology at that time, so botanists had to come to this conclusion from the study of plant materials, and zoologists had to come to the same conclusion from the study of animal materials. In 1824 a Frenchman, Henry Dutrochet, wrote that "the cell is truly the fundamental part of the living organism." But because the boundaries of plant cells are much easier to see than those of animal cells, it was not until 1839 that the idea was fully developed with respect to animals. Two Germans, Matthias Schleiden, a botanist, and Theodor Schwann, a zoologist, did much to popularize the generalization. As Schwann wrote, "We have thrown down a great barrier of separation between the animal and vegetable kindoms."

Thus, "cell," which once meant an empty space, came to mean a unit of living matter. Leeuwenhoek's "animalcules" were interpreted as the minimum cellular organization: a single cell. All other organisms were simply aggregations (groupings) of cells—very highly organized aggregations, to be sure, but all reducible to cell units. In the development of this generalization—the cell theory—we can see something of the international character of science.

But the cell theory did not lead to a great new era of research immediately. The detailed study of cells had to await another technological improvement—the development of dyes that could make visible their internal structure. This came with the great spurt in chemical knowledge that occurred in the 1850's and 1860's. Soon every life process was being linked to the cell. The cell came to be regarded not only as the unit of structure but the unit of function as well.

Already, in the decade after Schleiden and Schwann announced the cell theory, investigators began to find that cells normally and regularly come into being through the division of parent cells. Soon the idea was established that since the beginning of life there has been no break in the descent of living cells from other cells in the past, and that all of heredity and all of evolution must be embodied in cells.

The cell theory may be summarized as three wain ideas: (1) The cell is the unit of structure in living organisms. (2) The cell is the unit of function in living organisms. (3) All cells come from the division of pre-existing cells. Fig. 1.2. Generalizations,

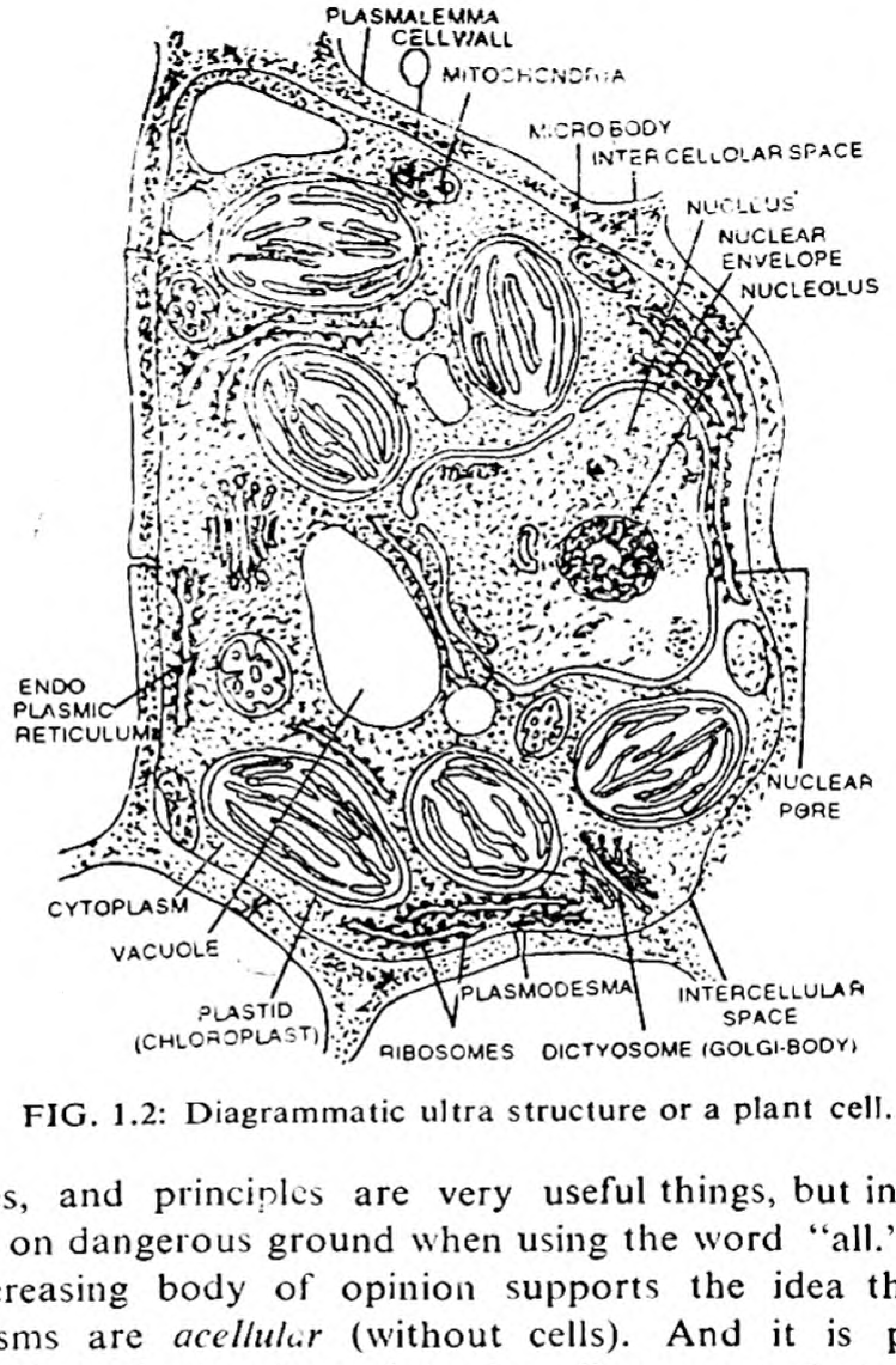

PLASMALEMMA
CELL WALL
MITOCHONDRIA
MICRO BODY
INTER CELLULAR SPACE
NUCLEUS
NUCLEAR ENVELOPE
NUCLEOLUS
ENDO PLASMIC RETICULUM
NUCLEAR PORE
CYTOPLASM
VACUOLE
PLASTID (CHLOROPLAST)
PLASMODESMA
INTERCELLULAR SPACE
RIBOSOMES
DICTYOSOME (GOLGI-BODY)

FIG. 1.2: Diagrammatic ultra structure or a plant cell.

theories, and principles are very useful things, but in biology we are on dangerous ground when using the word "all." Today an increasing body of opinion supports the idea that some organisms are *acellular* (without cells). And it is probably impossible to fit the viruses into the cell concept, but we may pass over them as being doubtfully alive. In some fungi, such as those of the genus *Rhizopus* the hyphae are made up of an undivided mass of protoplasm with many nuclei scattered about in it, whereas cells are usually thought of as containing one nucleus each.

USES OF THE TERM "CELL"

The term "cell" has, in its different uses, sometimes included and sometimes excluded the cell wall. Early students of plant anatomy, seeing cells only when the walls were thick and visible at low magnification, called these structures "cells", "pores," "bladders," and described their contents as nutritive juices. The walls were the prominent part of these "receptacles." When the cell content was recognized as the essential part of the cell, attention turned to it as "the cell." Following this recognition of the protoplast as the fundamental unit, the term "cell" was often used to designate the protoplast alone. In the more common sense, however, the term implies protoplast plus wall—these together constituting the obvious unit of structure (Fig. 1.3), a unit for which there exists no term if "cell" is restricted in use to the protoplast alone. The term is further applied even to units of wall alone, as, for example to the tracheid where the protoplast has disappeared, leaving within the walls a cavity, the *lumen*. The use of the term "cell" to indicate both protoplast and wall is desirable in view of the difficulties of expression otherwise involved and also in view of the intimate relationship between the two. The closeness of this

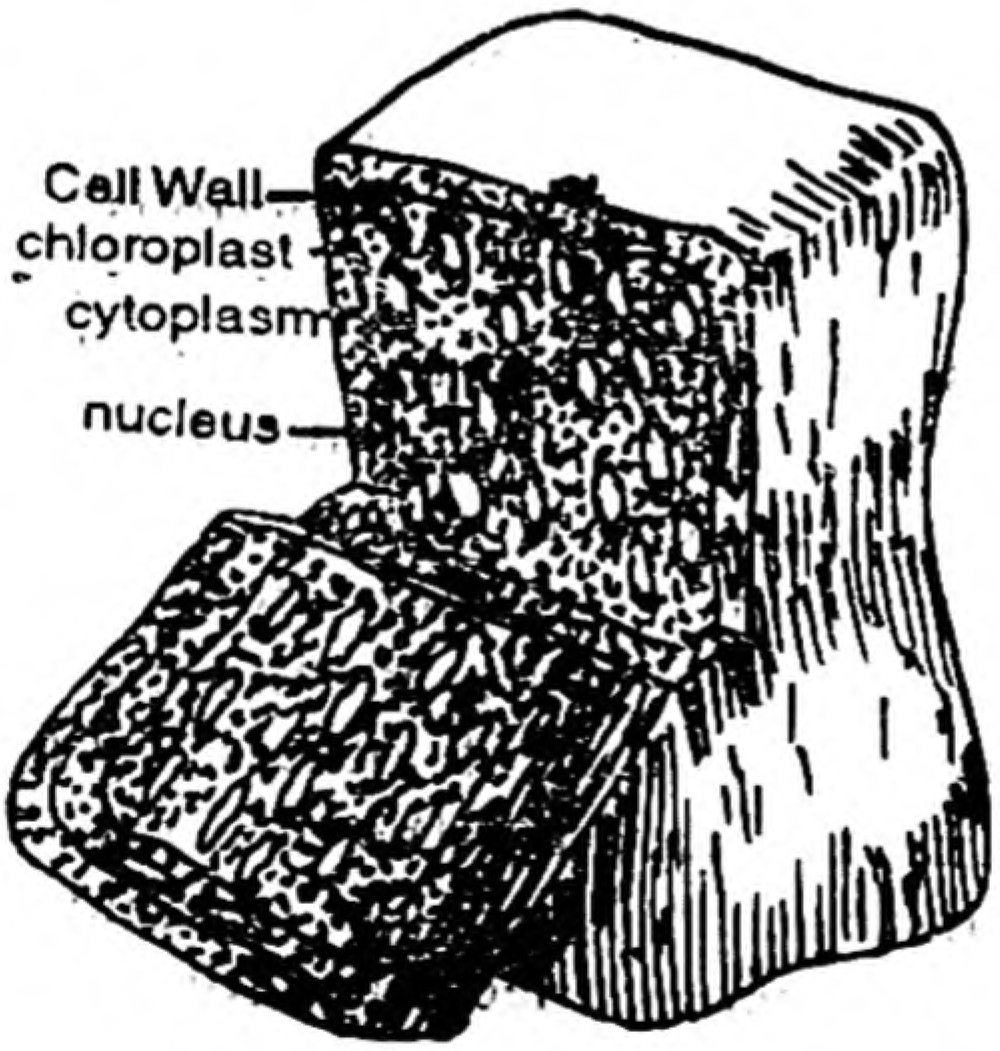


FIG. 1.3: Diagrammatic representation of mature mesophyll cell opened to show central vacuole (filled with cell sap), nucleus and plastids embedded in the peripheral cytoplasm; wall thin. (Based on Zea.)

relationship is not yet fully understood. Possibly the wall of a living cell is not merely a nonliving, external secretion of the protoplast, but is itself partly protoplasmic in nature. The use of the term to include both protoplast and wall is, of course, less strict than that unerlying the frequently used definition of the cell as "an organized and more or less independent mass of protoplasm constituting the structural unit of an organism." Nevertheless, from the standpoint of anatomy, the term is better used in the looser and more comprehensive sense.

The Cell Structure and Cellular Complexicty of Plants

The substance of the plant-body is not homogeneous, but, in all except the simplest forms, consists of aggregations or unions of microscopic structures, called cells, each *living* cell consisting of a day mass of a viscid substance called protoplasm, bounded by a distinct membrane, the cell-wall. These cells can be seen readily by teasing out the substance of a very ripe, mealy apple in water, and examining it under the microscope; also in thin sections of stems, roots, and other parts of plants viewed under the microscope. The protoplasm is the essential living substance of a cell, and the seat of vital processes. The cell-wall is formed by the activity of the protoplasm, and, according to the type of cell, is built up to perform special functions.

The cell-walls, therefore, are to be regarded as constituting a skeleton or framework, enclosing the living substance, and giving firmness and elasticity to the whole organism. They do not necessarily prevent communication between the living substance of the various cells, for the protoplasm of one cell may be connected with that of others by means of extremely delicate threads passing through the cell-wall. Thus the living cells of a plant are bound together in organic union, and carry on in harmony the processes necessary for the life of the plant.

In the simplest forms of multicellular plant, the organism consists of an aggregation of similar cells, all carrying on very much the same functions; but in higher forms, correlated with the physiological differentiation which has taken place, there is what is called Histological Differentiation. In other words, many different kinds of cells, more or less definitely arranged in groups, or tissues, can be recognised, the form and structure of the cells in each tissue being related to the functions which they perform. This differentiation becomes more and more marked as we pass from lower to higher types. In the Thallophyta and Bryophyta, all parts of the organism consist of *living* cells, although these cells may present many different forms. For this reason these two divisions are distinguished as "cellular plants."

A considerably higher degree of differentiation is exhibited by the Pteridophyta and Spermatophyta. These plants are rooted in the ground with their shoots exposed to the air, and possess elaborate arrangements for the conduction and distribution of nutritive substances. In addition to typical living cells of varied form, other cells are modified and allow the rapid transmission of fluids from the roots to the leaves or *vice versa*. Such specialised cells are usually associated in groups forming strands or bundles which supply the whole plant body, and are generally arranged in a definite pattern, according to the plant organ in which they are situated. These strands, concerned with conduction of fluids, are called vascular bundles and together they form the vascular system of the plant. The Pteridophyta and Spermatophyta are referred to as "vascular plants" because they possess these vascalar tissues.

Other cells may be modified in such a way that they give added strength to those organs which require it. Individual cells, or groups of cells forming a tissue, may be wholly given up to this function. Their cell-walls are strengthened, their form altered during development, and they may lose their living protoplasm, in which case they are dead. They are advantageously placed so as, for example, to give support to a stem which not only has to stand erect bear the weight of its leaves and branches, but has to withstand strains brought to bear on it by external influences such as wind. Such tissues are referred to as mechanical tissues.

As we proceed we shall see that certain tissues may perform more than one function. Thus a tissue may be vascular and also mechanical.

Cellular Complexity of Plants

The primitive organism was doubtless uncellular or, in a sense, noncellular. The larger body of more advanced types is multicellular and the cells are limited by walls. The walls perhaps separate regions of activity of individual nuclei but certainly serve also for protection and support to the individual protoplasts and to the entre organism. Apparently, this valed condition is of great importance in large plant bodies both from the standpoint of mechanical stability and of physiological delimitation. Clearly, adoptation to a terrestrial and aerial habitat and the maintenance of a large body under conditions unfavourable to protoplasm have resulted in the specialization of the cell along various lines, especially those involving elaboration of the wall. Hence, among higher plants there are many kinds of cells with much vareity in function, structure, arrangement, and with a great deal of complexity of wall structure. Variety, both structural and functional, in and of cell; variety in relation of cells to one another; variety in arrangement of cells and of systems of cells with relation to one another and to the body as a whole—these give great cellular complexity to the mon highly organized plants. Indeed, it may be said that in a general way the higher the plant, phylogenetically, the more complex its cellular structure

Cell Arrangement

Regularity of arrangement of cells is brought about by successive divisions in the same plane. Such arrangement is characteristic of secondary tissues (those formed by a cambium or similar meristem) and is occational in primary tissues, especially those of pith and cortex. But most primary tissues lack symmetrical arrangement and secondary tissues may lose this during development. Regardless of regularity or irregularity of arrangement and of the method of origin, a group of cells may be compactly arranged forming a close tissue continuous in one or more planes or they may be more or less free from one another, with the resulting formation of ***intercellular spaces*** between them. Such spaces vary in shape with the shape and arrangement of the surrounding cells, in continuity, in abundance, and in size from microscopically minute openings to very large spaces which render the tissue in which they lie loose, spongy, and light. Such large intercellular spaces are known as *air chambers*, *canals*, *lacunae*; etc. These terms are applied rather loosely—the larger spaces being called chambers and the much elongated ones canals. Spaces formed by the separation of walls followed by the retraction of the separated parts or by spatial movements of the cells said to be *schizogenous*; for example, the resin canal of *Pinus* and the spaces of the aerenchyama of *Decoden*. Others, developed by the destruction of cells formerly occupying the position of the cavity are called *lysigenous*; for example, the oil cavities of citrus fruits. Some cavities are formed by a combination of these two methods and have been called *schizolysigenous*. Some protoxylem lacunae are of the last type. The intercellular spaces of a region may be without uniformity in size and shape or in arrangement of the cells surrounding them; they may, on the other hand, form definite structural features of a tissue or organ because of the development about them of specialized limiting or supporting "walls," or layers of cells, such as the diaphragms of many aquatic plants. Intercellular spaces commonly form definite systems and constitute, perhaps, functionally conducting or aerating systems. Special types of intercellular-space systems form ducts and canals with cells regularly arranged about them forming a sort of epithelial lining.

Cell Shape

Because protoplasm is of semifluid nature, cells when free and independent tend to be spherical. But where young cells of the same type and essentially the same age lie together and grow in size, mutual contact and compression render them polyhedral with diameters nearly alike. Further growth toward individual or group specialization in relation to specific function leads to great variety in form—avoid, ellipsoid, cylindrical. tabular, prismatic, lamelliform, fibre-like, and stellate or otherwise lobed or branched. There are, however, two chief types: the *subglobose*, or *polyhedral*, with diameters equal or only slightly different; and the *elongate*, with one diameter many times that of the others. Transitional types are, of course. numerous.

Undifferentiated or unspecialized cells, in continuous mass. when surrounded and closely compressed by other cells of similar type and size, are on the average 14-sided. They approach in shape the orthic tetrakaidecahedron, a mathematically determined form for bodies of uniform volume and minimal surface that fill space without interstices. This form has 14 surfaces or contact—8 hexagonal and 6 quadrilateral. In plants. uniform cells of this shape are probably never formed. The closest approach is perhaps in parenchyma cells of pith that are arranged in columns. The surface cells of a mass of such cells have an average of 11 faces. Epidermal cells, for example, have about that number of contact faces. When intercellular spaces are present, the number of sides is less—the larger the spaces. the fewer the sides in contact with other cells. Where similar cells of different sizes lie together under these conditions, the larger cells have more than 14, the smaller less than 14 faces. The shape of newly formed cells is dependent upon that of the mother cell and upon the plane and frequency of division.

Cell Size

Cells vary greatly in size; size, like shape, is in part related to function. Extremely small cells do not occur among the higher plants. Parenchyma cells serving the usual functions, with normal protoplasm, have a transverse diametre of 0.01 to 0.1 mm. In pith and fleshy fruits, the diametre of parenchyma cells may reach 1 mm or more, and the cells then become readily visible to the naked eye. Fibres of wood and phloem range in length chiefly from 1 to 3 mm in the angiosperms, and from 2 to 8 mm in the gymnosperms. Cortical, pericyclic, and primary-phloem fibres are often much longer, and—partly because of their considerable length—are of particular economic importance, as, for example, those of flax and hemp. Fibres of excessive length—20 to 550 mm—occur in the Urticaceae and in certain monocotyledons. The largest cells known are latex cells of the type which form branching systems throughout a plant body. However, such cells are perhaps not individuals morphologically, since they are coenocytic and continue to grow almost indefinitely. Latex vessels consist of cells united ontogenetically in series.

Cell Development

All cells are formed from preexisting cells—or from nucleated protoplasmic masses—by division. Cell division is a complex process during which the nucleus and cytoplasm are each divided into two parts, usually equal and alike. In this division the wall is not directly involved. In the newly formed cells plasma membranes and intercellular substance are not readily distinguishable, and hence a visible plane of separation is at first lacking or scarcely detectable. But very early, new walls appear along this plane as delicate membranes. Cells, therefore, while still very young, are provided with walls. The small daughter cells soon enlarge to about the size of the mother cell.

Young cells are of more nearly uniform size and shape than mature cells and are simple in structure. Elaborate shape, complex structure, and very large size are not found among young cells. Growth of cells involves increase in size and the development of special shape and structure. Since the wall is present from a very early stage, both protoplast and wall are involved in these changes. As the active part of the cell, the protoplast initiates the changes, the wall being modified with the changing protoplast. While changes in size and shape are taking place, the physical and chemical nature of the wall is usually such that these accommodations are readily made; only after cell maturity is attained is a fully developed and unchanging wall present. In the maturing of the protoplast many changes occur. These are, briefly, the reduction of the proportionate size of the nucleus; the development, usually, of less dense or less richly granular cytoplasm; the appearance of vacuoles that increase in size, and, in most cases, ultimately fuse to form one large central vacuole which restricts the cytoplasm to a peripheral position adjacent to the wall; changes in size and type of vacuole, where vacuoles are already present in the mother cell, for example, in cambium initials and their daughter cells; the increase of plastids an size and number, and the development of special types of plastids. In the maturing of the wall there occurs increase in extent and thickness by accompanied complexity of form and changes in chemical and physical structure.

Cellular Adjustment During Growth

Growth frequently brings about changes in the relationships of contact and position among neighbouring cells. The changes may involve all surfaces of a cell or be localized. In many cells the rate of growth of the wall is unequal in different areas, and in areas of greater or more rapid growth there are commonly greater changes in intercellular contacts. Development of elaborate or extreme shape and size—especially in such cells as large vessel elements, fibres, and some types of sclereids —brings about great changes. These may involve the deep penetration of cell tips or lobes between adjacent or nearby cells and into intercellular chambers. The steps in these changes are not fully understood. As new contacts are made by a penetrating tip and cell walls are separated, the intercellular cementing substance must be modified or removed. The plasmodemata ol the region are ruptured and the primary pit-fields and even partly developed pit-pairs are divided.

There is lack of agreement as to the manner in which these new wall contacts are made. *Gliding* (or *sliding*) *growth* has commonly been described as the basis of these changes. *Intrusive growth* and *symplastic growth* have recently been proposed as supplanting gliding growth in part or wholly. None of these three methods alone seems to cover the conditions met in all tissue and cell types.

By *gliding growth* is understood the slipping, during growth, of the wall of a cell over the wall of a contiguous cell along the surface of contact so that new areas of contact are made with the same cell and with nearby cells with which no contact previously existed. It has been generally assumed that real movement takes place in this type of growth, that the wall is increasing in area over more than the points of new contact, sometimes throughout, and that a sliding therefore occurs. Examples of apparent gliding growth are to be found (*a*) in the formation of new cambium initials with increase in the girth of the cambium—size, form, and position of the new cells are evidence of growth throughout the cell walls and of slipping and the making of new contacts; (*b*) in the development from cambium derivatives of mature phloem and xylem elements—nonmatching pit-fields in the phloem and pits without complementary pits in the xylem are evidence here. The development of the lobes or arms of some types of sclereids is an example of gliding growth of parts of the cell wall.

Gliding growth has also been assumed to cover extreme changes in cell contacts brought about by the forcing, under pressure, of cells into new positions and distorted forms. As an example of this, the distortion in developing secondary xylem of cell arrangement and form about enlarging vessel elements has been cited. In ring-porous woods such as that of *Quercus*, the rapid and very great increase in diameter of a vessel element results in compression, or stretching, ever tearing apart, of the smaller cells nearby. These cells find new positions, and their new contacts must often involve large areas of their wall surfaces. A sliding of cells on one another is the obvious method of change. Continuing growth cannot explain so great a change of position. It has been claimed that these changes are the result of cell multiplication among the smaller cells, but no cell division is found at this stage and the additien of new cells in the area would not explain extreme distortion of form.

By *intrusive growth* is understood *differential* expansion of the wall—the increase of cell size locally, with the projection of the newly formed parts of the cell between adjacent cells or their extension into intercellular spaces. The parts of other cells with which there is new contact may or may not also be enlarging. In this type of change it is understood that no real slipping occurs; the new contacts of a cell are those made by new areas of the wall as they form. The elongation of the apices of fibres, trachcides, and vessel elements has been called intrusive growth, but it is perhaps gliding rather tean intrusive growth, or, more likely, it involves changes of both these types. An example of extreme continuation of intrusive growth is found in the latex cells of the milkweeds—Apocynacease and Asclepiadaceae—whose rapidly growing apices maintain their position in apical meristems throughout the plant.

Most gliding and intrusive growth undoubtedly occurs while the cells are very young, but some apparently takes place after the walls are partly mature. In secondary xylem, walls of contiguous cells, already so mature that secondary walls and the beginnings of pits are present, may be split apart by the developing tips of adjacent partly mature cells. Such pits become functionless and are present in mature cell as *blind pits* (pits without complementary pits in the adjacent cell wall). Blind pits may even lead toward an intercellular space; the matching pit may be found across the space. (In sections pit-pairs often appear to extend only part way through the wall; these are usually parts of a pit-pair cut obliquely). Intrusive growth may bring about a wrinkling of the wall of the intruding cell tip.

By *symplostic growth* is understood growth in size throughout a group of young cells with mutual adjustment in shape by all members of the group. The entire walls are growing. New shapes are formed and new spatial positions taken, but there are no new contacts and no movement either gliding or intrusive. The group of cells grows thoughout with their walls forming a framework that is continuously adjusted under pressure and tension. Diffierences in rate of division and rate of enlarge ment play a prominent part in symplastic growth. Growth of the derivatives of the initials in an apical meristem is an example of symplastic growth.

All three types of growth doubtless occur. Symplastic growth is characteristic of groups or masses of cells in early stages of growth. Evidence in support of the occurrence of typical gliding growth in many tissues is strong. Distinction between gliding and intrusive growth is doubtless one of degree rather than kind—the difference depends upon the definition of "tips" of the the cell. If only a small part of the cell at the apex is growing and making new contacts with the walls of other cells, growth is intrusive; if there is growth in regions other than the tips, there must be movement of the gliding type.

THE YOUNG CELL

Cells arise from pre-existing cells by a process of cell-division. All plants begin life as a single cell. They may remain unicellular, or become multicellular as the result of repeated cell-division. As we pass from the lower to the higher plants, along with the increasing size and complexity of the plant-body, we find that cell-division becomes localised. All living cells are potential dividing cells, but cells which have become specialised for various functions do not usually divide further unless stimulated to do so, as by wounding. In the higher plants dividing-cells are found, particularly at the tips of roots and stems, which are consequently termed the growing points. In members

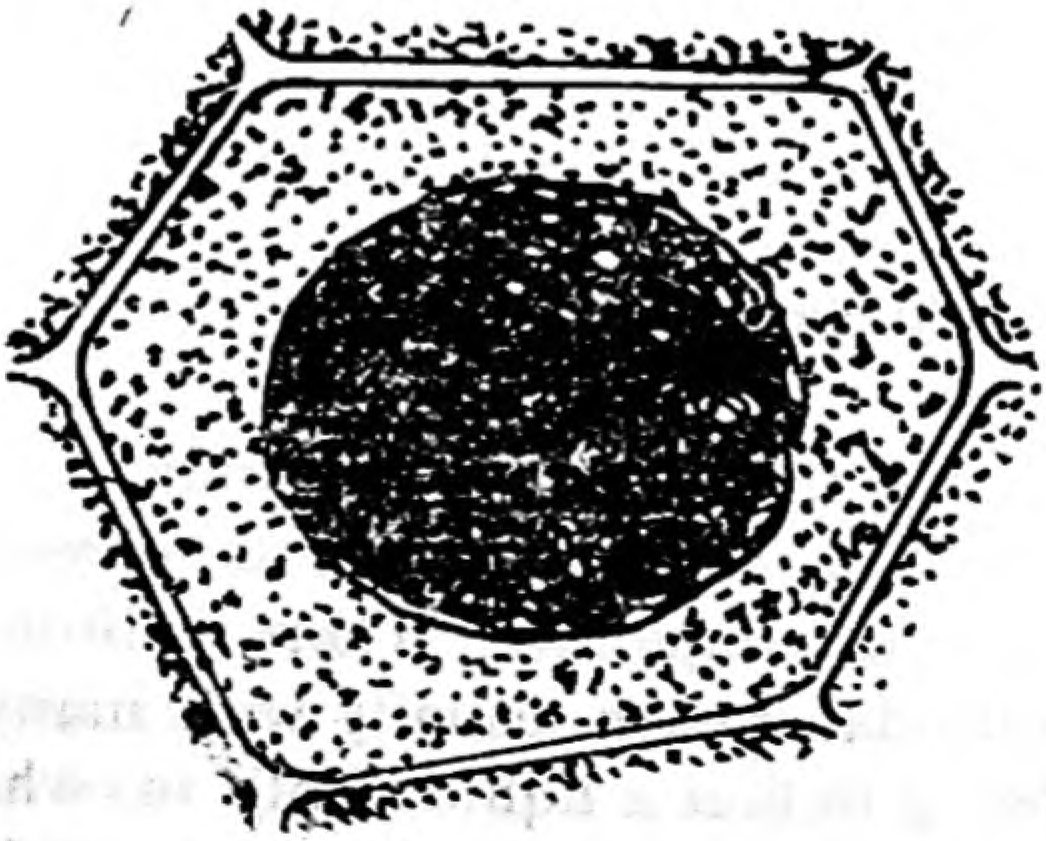


FIG. 1.4: Meristematic Cell, Resting Condition.

of the Bryophyta and Pteridophyta a single apical cell of definite form may repeatedly divide. In the Spermatophyta two or more layers of cells may be concerned. Such cells are said to be meristematic, and the growing points primary meristems. Secondary meristems may arise later in living cells which have originated from the primary meristems.

The growth of the multicellular plant-body depends, in the first place, on cell-division, then on the enlargement of the cells thus produced; and their differentiation and adoption of various functions.

In order to examine the structure of a meristematic cell it is necessary to cut thin sections, e.g., of a root-tip, and examine them under the high magnification of a compound microscope Fig. 1.4. Transverse and longitudinal sections are required to give a complete picture of the individual cell. In longitudinal section it may appear more or less rectangular, and in transverse section it may be rectangular or several-sided. In the solid, therefore, it is shaped something like a prism or truncated pyramid with four or more sides, the short axis parallel to the axis of the root.

The meristematic cell is bounded by a thin cell-wall and contains the protoplast. The latter consists of a relatively large central nucleus and the surrounding cytoplasm. Within the cytoplasm are suspended specialised protoplasmic bodies called plastids or chromatophores, and non-living inclusions in the form of minute granules and droplets.

The Protoplasmic Substance

Observation of the living cell indicates clearly that the *protoplasm* is not homogeneous, but its physical nature is still rather a matter for conjecture. There is little doubt, however, that it is a colloidal system, usually with many of the properties of a liquid, being in fact a liquid (sol) in which are dispersed either liquid or solid particles which are either aggregates of small molecules, or are very large molecules. The particles dispersed in the continuous liquid phase of the protoplasm are extremely minute, and often only just visible with the most powerful microscope. Many of the properties of the protoplasm are the outcome of its colloidal nature.

Chemically, the dry substance of the protoplasm appears to consist mainly of proteins with appreciable amounts of fats and fatty substances and of carbohydrates, and smaller amounts of other organic compounds and of mineral matter. Analysis of the protoplasm always, of course, includes any substances present in it at the time, that is, both living and non-living protoplasmic inclusions. But it must be remembered that methods of chemical analysis bring about the death of the protoplasm, and so the analysis is really of *dead*, and not living protoplasm.

The analyses do, however, show the presence of substances (fat-like compounds and proteins) which it is known are capable of forming the disperse phase in a colloidal system. The evidence of chemical analysis and to observation of the physical properties of the protoplasm suggest that in the protoplasm we have a continuous phase of water containing proteins and fatty substances, and dispersed in this are granules (solid or liquid) of protein, either alone or united with other substances, the whole being of the consistency of a slightly viscous liquid.

The surface layer of the protoplasm differs from the inner part, and in a system such as that outlined above we might expect to find in the outermost layers a concentration of fatty substances. Any such surface layer is certainly of a complex nature and of vital importance, as its properties will govern the entry of substances into, and their exit, from the protoplasm.

Protoplasm is a highly complex mixture of some elements and compounds found in the bodies of living beings. The protoplasm is variously known as the *living matter, living substance* or the *physical basis of life.* It is the basic fundamental substance exhibiting all the vital processes of the cell. The protoplasm was first observed by *Corti* in 1772. In 1835 *Dujardin* a Frenchman described it as a soft and gumy substance and named it as *sarcode i.e., flesh. Purkinje*, a Bohemian physiologist (1839), was the first biologist, who gave the name *protoplasm* to this living substance. *Hugo von Mohl*, a German botanist, in 1839, also suggested the name protoplasm for the granular and viscous substance found in plants similar to that found in the animals. The above-mentioned German botanist popularized the word protoplasm as a name given to the living matter found in plants and animals. Protoplasm is the most complex and interesting substance. It is not to be thought of a chemical compound but rather as very complex organized system. Protoplasm varies somewhat in its nature from cell to cell and from organism to organism, but basically it must be the same, as evidenced by its common manifestations of metabolism, growth, reproduction and by some other peculiarities.

Physical Nature of Protoplasm

Different workers have proposed different theories to explain the physical nature Fig. 1.5 of protoplasm as given under the following heads:

1. *Granular Theory.* This theory was propounded by *Altmann* in 1893. According to this theory, protoplasm consists of numerous tiny granules as shown in *Amoeba, Henle, Maggi*, etc., considered these protoplasmic granules as *plastidules. Altmann* recognized them as "elementary oraganism", or *bioplasts*, (or cytoplasts).

2. *Alveolar Theory.* The alveolar nature of protoplasm was suggested by *Butschilii* in 1892. According to him, protoplasm consists of many suspended droplets or alveoli or minute bubbles, resembling the foams of emulsion.

3. *Fibrillar Theory.* This theory was put forward by *Flemming*. According to him, protoplasm consists of fibres embedded in the inner mass of matrix. The fibrillae are called mitome or spongioplasm and ground substance is temed paramitome or hyaloplasm.

4. *Reticulate Theory.* This theory was proposed by *Klein, Carnoy* etc., suggesting that the protoplasm consists of a network or reticulum of fibres in the ground substance.

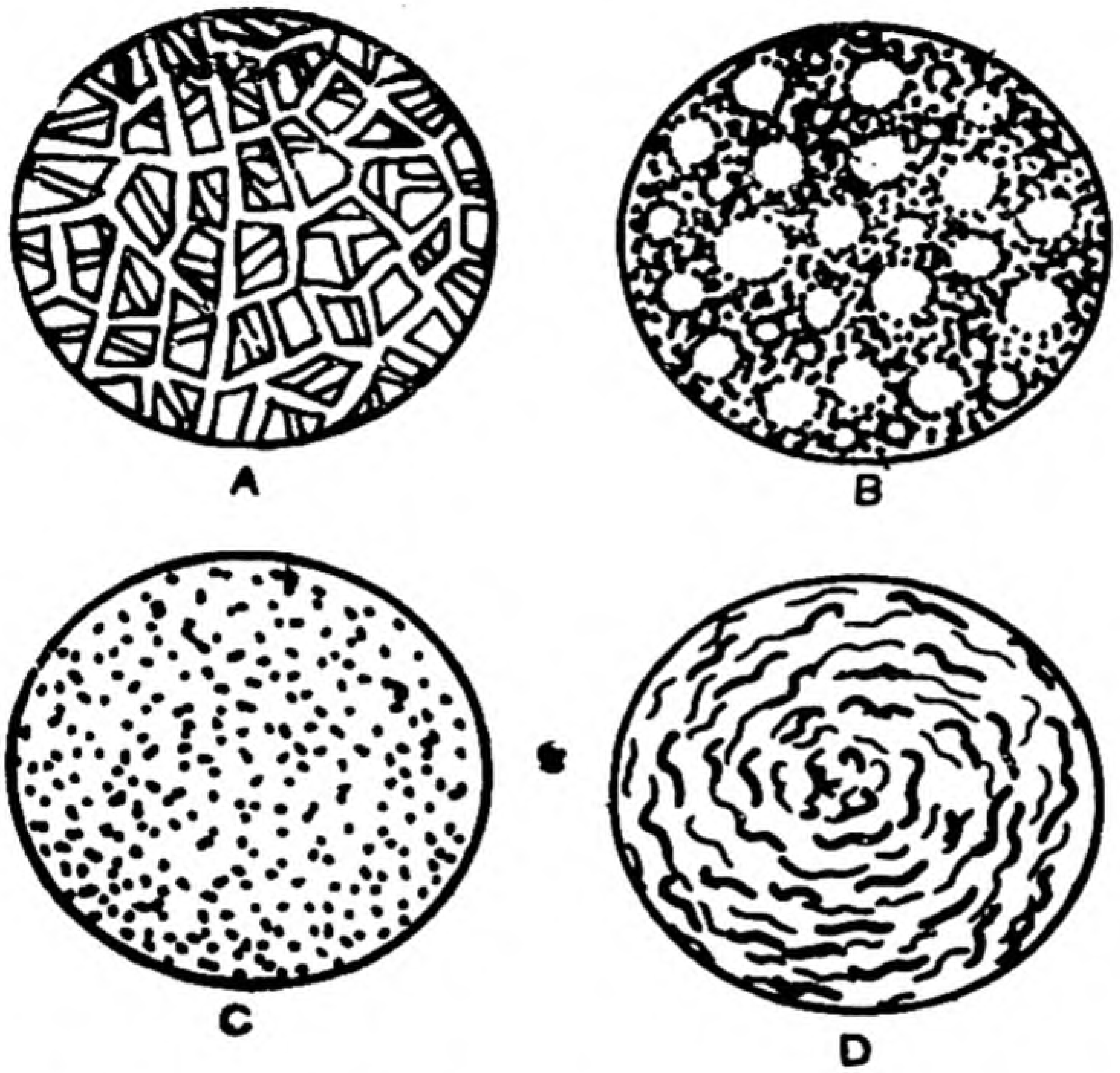


FIG. 1.5: Physical appearance of protoplasm. A. Reticular B. Alveolar C. Ganular D. Fibrillar.

5. *Colloidal Theory.* Proposed by *Wilson* in 1925. According to this theory the protoplasm having any of the aforesaid appearances is always a fluid-colloidal system having various chemical inclusions in *gel phase*. A gel is a semi-solid condition which presents jelly-like appearance. Here the molecules are held together by various bonds depends upon their nature and strength. By absorbing water the gel changes to more liquid-like phase. This is known as *sol* and the process is termed as *solution.* The sol can stream easily and by losing water again changes into gel-state. Bol these states of colloidal matrix are interchangeable according to the various physiological, mechanical and biochemical activities of the cell. This represents phase reversal in the colloidal system. These sol-gel

conditions of colloidal system are the basis for the mechanical behaviour of protoplasm.

Properties of Protoplasm

1. *Cohesiveness.* The various particles or molecules of protoplasm are adhered with each other by forces, such as Van der Waal's bonds, that hold long chains of molecules together. These Van der Waal's bonds are weak and non-specific forces between non-polar groups of atoms. This property varies with the strength of these forces.

2. *Contractility.* This property is significant in various stomatal operations in plants. The contractility of protoplasm is important for the absorption and removal of water as they generally occur in protoplasm.

3. *Electrical charge.* Protein molecules repel each other because their overall charge is similar. All molecules are either positively charged or negatively charged. However, if the molecules appraoch one another close enough so that valency forces can act, then they may be attraced to each other.

4. *Precipitation.* Addition of certain amount of electrolyte in a colloidal suspension causes its dispersed particles to colloide. aggregate and finally to precipitate as suspension. For example, the addition of HCl to a colloidal system of arsenic sulphide causes precipitation.

5. *Viscosity.* The viscosity of the ground substance of the cell varies greatly. It may be as low as that of water, or may be very high in the gelating cytoplasm of pseudopodia of *Amoeba*.

6. *Streaming movement or cyclosis.* The protoplasm exhibits various sorts of streaming movements inside the cell. These have been studied in *Gleve cluox Amoeba*, *Paramecium* etc. The movement involves only the localized portions with no visible changes in the protoplasm. No complete explanation of this

has been given as yet. But it is seen that its rate depends upon the rate of cell metabolism. It is due to the fact that the energy is supplied by respiration.

7. *Amoeboid monement.* The amoeboid movement as exhibited by *Amoeba* and other protozonans involves the movement of entire protoplasm of the cell, where the cytoplasm moves as one mass carrying the various inclusions with it. It is due to the continuous change of gel to sol and sol to gel.

8. *Brownian movement.* It is characterized by the zigzag motion of suspended colloidal particles, occurring due to the bombardment of one particle or molecule by other. This type of movement of particles was first of all observed by ***Robert Brown*** in 1827 in the colloidal solution and hence such movements are known as Brownian movements. The higher the temperature, more rapid the movement and thus viscosity of cell is decreased. This means that high viscosity indicates a more gel-like state of protoplasm and low viscosity, a more sol-like condition.

9. *Tyndall effect.* Colloidal particles of protoplasm have the property of scattering light. When a beam of light is passed through a colloidal solution it becomes visible. This is the Tyndall effect. A colloidal solution of proteins in water shows a typical tyndall cone.

10. *Adsyrption.* The tendency of particles, molecules or ions to adhere to the surface of certain solids or liquids is known as adsorption and is exhibited by the particles of colloidal system. The phenomenon helps the matrix to form protein boundaries.

11. *Biological properties.* Protoplasm has all the biological properties of a living organism. It is capable of nutrition, respiration, excretion, metabolism, growth and reproduction. It has the property of irritability, *e.g.*, it responds to stimuli like heat, light and chemicals. It also has the property of conductivity, *i.e.*, of conducting impulses produced by stimuli.

Chemical Nature of Protoplasm

For the detailed study the chemical components of the cell can be classified as inorganic (mineral salts) and organic (proteins, carbohydrates, nucleic acids, lipids and so forth) substance. Although the most prominent costituent of protoplasm is water—the substance which gives protoplasm its characteristic structure is protein. Lipids are important in all membranes and carbohydrates serve as nutrient stores. The protoplasm of a plant of animal cell contains 75 to 85 per cent water, 10-20 per cent protein, 2-3 per cent lipids, 1 per cent carbohydrats and 1 per cent inorganic material. The following Table 1.1 gives approximate figures of the relative amounts of the main inorganic and organic compounds found in active protoplasm.

TABLE 1.1

Substance	*Per cent*	*Average molecular weight*	*Number of molecules in relation to protein*
Water	85	18	180
Protein	10	36000	1
DNA	0.4	10^6	—
RNA	0.7	4.0×10^4	—
Lipid	2	700	10
Other Organic master	0.4	250	20
Inorganic substances	1.5	55	100

Water. Water is the main component of protoplasm and occurs in large amount, about 85 per cent. It serves as a natural solvent for other materials and also plays a major role in the metabolic activities, since physiologic processes occur exclusively in an aqueous medium. The water molecules also participate in many enzymatic reactions in the cell. No doubt, certain amount of water is formed in the cell as a result of metabolic processes, but this is insufficient to maintain the water balance of the body. Therefore, it must be and is supplied from the outside. Inside the cell, the water exists free, *i.e.*, a solvent as well as bound, *i.e.*, tied to polar groups of protein molecules by hydrogen bonds.

Inorganic Compounds

A number of inorganic salts occur both in free as well as in the ionised state. The elements that occur in quantity in protoplasm are oxygen, carbon, hydrogen, and nitrogen. Always present but in much smaller amounts are potassium, phosphorus, calcium, sulphur, magnesium and iron. These ten are generally referred to as *essential elements.* Chlorine and sodium

TABLE 1.2

Showing Percentage by Weight to the Elements in Protoplasm

Elements	*Symbol*	*Per cent*
Oxygen	O	65.04
Carbon	C	18.24
Hydrogen	H	10.05
Nitrogen	N	3.15
Potassium	K	1.60
Phosphorus	P	0.84
Calcium	Ca	0.25
Sulphur	S	0.20
Magnesium	Mg	0.04
Iron	Fe	0.01
Chlorine	Cl	0.27
Sodium	Na	0.26

are necessary components of most animal protoplasm but are apparently not essential for plant protoplasm. Copper. boron, iodine. maganese, zinc and several other elements which are present in all protoplasm but only in minute quantities, are called *trace elements.* This term must be used with care, because these elements are still important even though they are present only in small quantities. The essential elements found in human protoplasm are listed below in Table 1.2.

The elements are almost always present in protoplasm in the form of chemical compounds rather than elements. Many of these compounds are inorganic salts, which are usually in solution. These salts have numerous functions. They serve as sources of elements to be built into other compounds, and some act as buffer in maintaining the proper acidity or alkalinity in the protoplasm. Because salts in solutions are electrical conductors, they also function in some way in connection with the electrical properties of protoplasm, however, this is not well understood at present.

Organic Compounds

Proteins

Proteins are the framework of protoplasm. About 10 per cent of protoplasm is protein. Proteins are linear polymers of high molecular weight. All proteins contain C, H, O and N, the presence of N distinguishing them from carbohydrates and fats. On an average proteins contain 16 per cent nitrogen. Some poteins also have S in addition, and in a few proteins P and other elements may be per cent. The molecular weight of proteins varies from about 12,000 daltons (bovine insulin) to several million. Cells contain a very large number of proteins. The number may vary from 1000-2000 in the simplest bacteria to as many as 100,000 different proteins in human cells.

Protein are found in two form: Simple and conjugate.

The simple proteins are compounds, which on hydrolysis, yield exclusively alpha (α) amino-acids. In this group the most important proteins are the albumins (soluble in water and coaguable by heat; the globulins (insoluble in water but soluble in dilute salt solutions); the protamines, strongly alkaline components of the sperm cell (*e.g.*, clupein, salmine, etc.); and the some what less basic histones which are found in many cell nuclei.

Conjugate proteins are a combination of a simple protein and another substance called the *proshetic group*. Unlike the simple protein, the conjugate protein yields on hydrolysis alpha (α) amino-acids and an organic component. Nucleoprcteins are also included in conjugate protein group which play in important role in the cell and whose prosthetic groups are the nucleic acids; the glyco-protein (mucoproteins) in which protein is combined with a carbohydrate; the lipoproteins, which are combinations of proteins with higher fatty acids; and the chromoproteins, a very widely distributed group, included a series of substance of great biological importance which are characterized by their particular colours. Among the chromoproteins are haemoglobin, in which the globulin is combined with an iron-porphyrin compound, and the haemocyanin which occurs in the blood of various invertebrates and in which copper is found in a position similar to that of iron in haemoglobian. A series of respiratory enzymes (cytochromes, flavopratein etc.,) belong to the chromoprotein group.

Significance of Proteins

Proteins are the most significant compounds in the body of living organisms because of the following physiological roles performed by them:

1. ***Proteins confer specific individuality to living structures, organisms and the species.*** This is known as biochemical individuality—Protein individuality is well illustrated by blood transfusion experiments. The blood of one species of animals cannot be transfused into the circulation of animals of other

species. Not only this, the blood proteins of different individuals of the same species are not identical.

2. *Proteins play an important role in life proceses.* These can combine with both acid and bases and can neutralize excess of any one of them in the protoplasm. These check the accumulation of H+or OH—ions in the protoplasm.

3. *Proteins have a high chemical potentiality* and, therefore, readily react with many substances, therefore, these act as enzymes and catalyse almost all the life processes of living organism.

4. *Proteins form boundaries of living cells* and their components and thus do not permit an easy access to and from the cell cytoplasm.

5. *Protein differentiation of organs and animals.* How only twenty amino acids lead to the formation of thousands of proteins is a complex but interesting question. For easy understanding of this phenomenon the twenty amino acids are linked to 20 alphabets of English language and the formation of a protein with the construction of a word. For example, an insulin molecule formed of 16 amino acid molecules is similar to a word formed of sixteen letters. In English dictionary 26 alphabets combine to form at least 2,00,000 words. Similarly 20 amino acids can give approximately same number or even more varieties of proteins. Such a wide variety of proteins will naturally bring wide range of differentiation.

Amino Acids

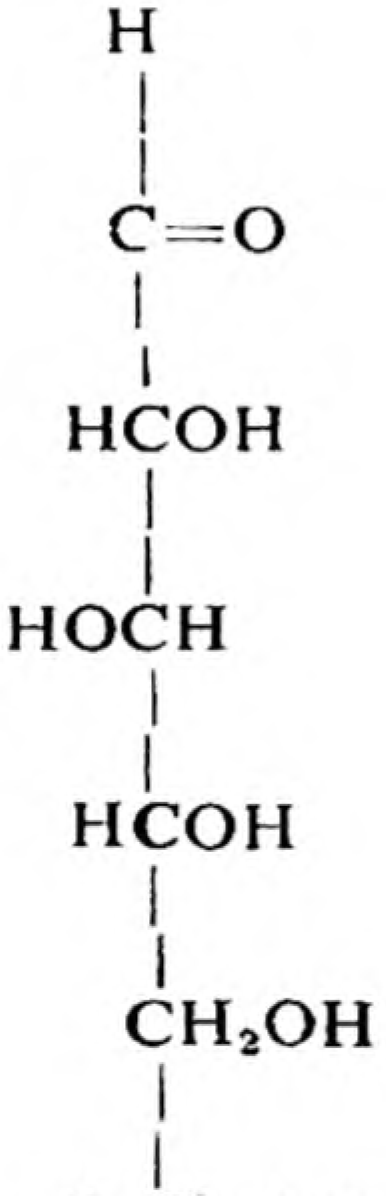
There is a common plan of construction for the thousands of kinds of proteins in living systems. The 20 kinds of naturally-occurring amino acid monomers are strung together in unbranched, linear polymer chains of proteins. These are the 20 amino acids specified in the genetic code that is universal to all organisms. Some other kinds of amino acids are also found in cells, but they are either degradation products or residues that have been modified from one of the 20 commonly occurring amino acids after this latter has been inserted into the polymer chain. Hydroxyproline is a major amino acid constituent of collagen in connective tissue, but proline residues are initially included in the protein and are converetcd to hydroxyproline after polymerization. Hydroxyproline is not one of the encoded amino acids, but proline is. Many proteins contain fewer than 20 kinds of amino acids. The relative proportions and the absolute numbers of the amino acid repertory vary from one protein to another, as a reflection of the specific in formation in genes, which are the blueprints for protein construction.

Carbohydrates

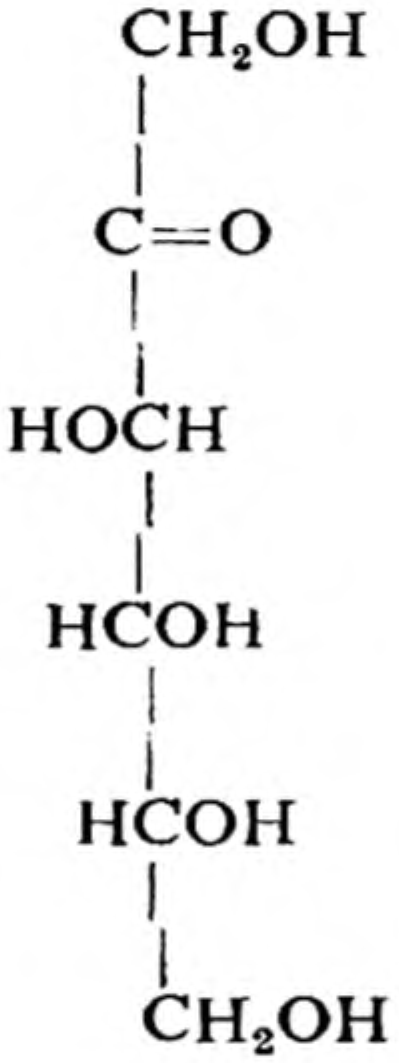
The carbohydrates (*L.*, *carbo*-carbon or coal, *Gr.*, *hydro*-water) are the compounds of the carbon, hydrogen and oxygen. They form the main source of the energy for all living beings. Only green parts of plants and certain microbes have the power of synthesizing the carbohydrates from the water and CO_2 in the presence of sunlight and chlorophyll by the process of *photosynthesis.* All the animals, non-green parts of the plants *viz.*, stem, root, etc.), non-green plants (*e.g.*, fungi), bacteria and viruses depend on green parts of plants for the supply of carbohydrates.

Carbohydrates can be classified into:

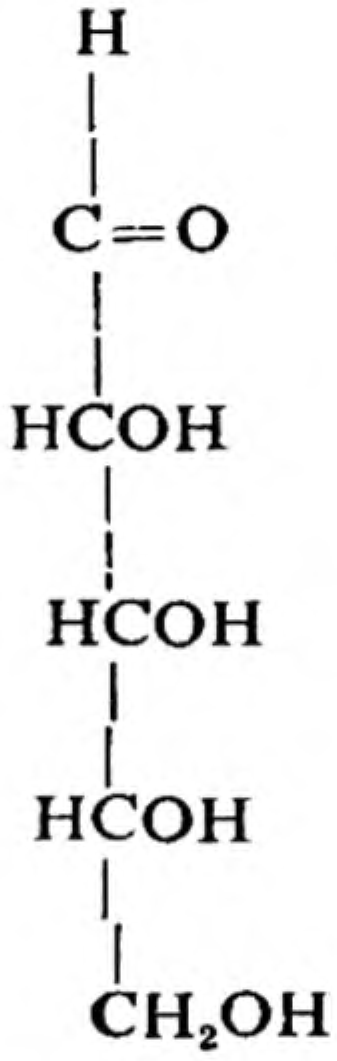
Monosaccharides. These are simple sugars with an empirical formula $Cn\ (H_2O)n$. These may be of trisoes if they have 3 carbons, tetroses, if they have four carbons, pentsoses type, if they have five carbons, or hexoses, if they have six. Glucose or grape sugar and fructose of fruit sugar are examples of hexoses type. And ribose and deoxyribose (the sugars present in nucleic acids) are examples of pentose. The monosaccharides are readily soluble in water and can pass across the membrane and can also be crystalized. This soluble nature is changed, when these are stored in the animal as well as in plant tissues. In such cases it is usually polymerized to an insoluble form such as glycogen (animal starch) in animals and starch in plants.



D Glucose



D fructose



D Ribose.

Disaccharides. The disaccharides are having the empirical formula $C_{12}H_{22}O_{11}$ and are readily soluble in water and these can also be crystalized and passed through the membrane. The commonest and best known disaccharide is sucrose or table sugars which is stored in such plants as the sugar cane and the sugar beet. On hydrolysis yields monosoaccharides or simple sugars.

Polysaccharides. These are formed by the condensation of many molecules of monosaccharides with a corresponding loss of water molecules. Their empirical formula is $(C_{16}H_{10}O_5)n$. They yield molecules of simple sugars on hydrolysis. Biologically important polysaccharides are the starch, the glycogen, and the cellulose. The former two are the reserve substances in cells of plants and animals respectively where as the cellulose is the characteristic structural element of the plant cells. The starch $(C_6C_{10}O_5)n$ is a mixture of two long polymer molecules, *i.e.*, linear amylase and branched amylopectin. It has no structural importance in animals. The glycogen $(C_6H_{12}O_6)n$ is generally called as the starch of animal cells. It is a polymer composed of many molecules of glucose and thus represents an important reserve of energy in the body. Though found in numerous tissues and organs, but contained in liver cells and muscle fibres in greatest proportions. It forms a colloidal solution in the protoplasm. The cellulose is composed of hundred of monosaccharides. It has no structural importance in animals except the tunicates which have a wall of cellulose. It takes part in the formation of cell wall and also a series of other structures which form the supporting skelecton in plants.

Besides cellulose, plant tissues contain several other structural components such as xylen. alginic acids (in algae), pectic acid, etc.

Significance. Thus we can conclude that carbohydrates play three main roles in the cells. These are:

1. They furnish the most readily available fuel.
2. They are important articles of storage.
3. They have a minor role in furnishing part of the structural material of atleast one part of the essential environment of living cells.

Lipids

Lipids comprise a rather heterogeneous class of compounds which are sparingly soluble in water, but show considerable solubility in organic solvents like ether, chloroform, benzene, hot alcohol and petroleum ether. Their physical properties indicate the hydrophobic nature of the hydrocarbon structure, although lipids may also contain some hydrophilic groups.

Lipids have several biological functions:

1. Certain lipids *e.g.*, triglycerides are storage compounds for the reserve energy of the body, *e.g.*, lipids of the fat body and subcutaneous fat.

2. Lipids are important components of cell membranes. The plasma membrane of eukaryote cells is composed primarily of lipids and proteins, along with carbohydrates in some cases.
3. In nerve fibers the myelin sheath contains lipids which act as electrical insulators.
4. As components of subcutaneous tissue lipids are important for insulation of heat.
5. Some hormones *e.g.*, steroids are lipids.
6. Some lipids are important as vitamins.
7. Lipids also occur as components of some enzyme systems.

Lipids can be classified as follows:

(I) Simple lipids,
(II) Compound lipids.

I. Simple Lipids

Simple lipids are the esters of the alcohols or the triglycerides containing fatty acids and alcohols.

$$\text{Triglyceride} \xrightarrow[\text{H O}]{\text{Lipase}} \text{Glycerol} + 3 \text{ Fatty acids.}$$

The constituent fatty acids may be *saturated*, *e.g.*, palmatic, stearic acids, or *unsaturated*, *e.g.*, oleic, linoleic, arachedonic and clupanadonic acids. The lipids of the matrix of animal cells contain fatty acids, *viz.*, palamatic, stearic palmitoleic, oleic, linoleic and lnolenic acids. The simple lipids of the matrix are as follows:

1. *Natural fats* The natural fats are the naturally occurring fats which occur in animal and plant cells as stored food substances.

2. *Waxes.* Waxes are esters of higher fatty acid with alcohols other than glycerols. In the human body the commonest waxes are the cholesterol esters. They are most abundant in the blood, suprarenal glands, the gonads and the sebaceous glands of the skin. The natural waxes are solid at ordinary temperature and are not so readialy hydrolysed as fats. Three common waxes are as follow:

(a) Beeswax
(b) Lanoline
(c) Spermaccti.

II. Compound Lipids

These are the lipids which on hydrolysis yield other compounds, in addition to the alcohols and acids. They are of following types:

1. *Phospholipids.* Phospholipids are soluble in both water and fat solvents, and, therefore serve an important role in binding both types of compounds together. They contain in addition to fatty acids and glycerol; phosphoric acid and a nitogenous base.

Three types of phospholipids have been indentified:

(a) *Lecithin.* When placed in water, it swellss up and readily forms an emulsion. Due to presence of acid and basic groups, it combines both with bases and acids. Thus these are important as a structural material in the cell membrane since by its hydrophilic group it maintains the continuity between the aqueous outside and the aqueous inside of the cell; yet fat soluble material dissolves in it and enters the cell because of its hydrophobic group.

(b) *Cephalin.* It is similar to lecithin. Cephalin and lecithin occur together in the tissue. *Howell* claims that the substance thrombokinase which initiates blood clotting is identical with cephalin. It is found specially in nerve tissues, egg yolk, etc.

(c) *Sphingomyelin.* These are derivatives of sphingosinol. It is also found in nerve tissues, egg yolk etc.

(d) *Plasmalogen.* It is another type of phospholipid present in the brain, liver and muscle tissue.

2. *Glycolipids.* These are compounds of carbohydrates. fatty acids and sphingosinol which contain nitrogen, but no phsophoric group. They are also called as glycosphingosides. They are specially abundant in the brain.

3. *Gangliosides.* Found in nerve cells, spleen and red blood cells.

4. *Lipoprotein.* These are the complexes of lipids and proteins. Lipoprotein is present in cell membrane, egg yolk etc.

5. *Sulpholipids.* They are sulphuric esters of sphingosine cerebronic acid and galactose. Sulpholipids are observed in white matter of brain and to a lesser extent in liver, kidney, salivary gland, testes, etc.

III. Steroids

Steroids are not true fats. These are characterized by OH group thus are called steroils and are of highly importance for the body. Male and female sex hormones, adrenocortical hormones, vitamin D, cholesterol are major steroids. They are found in cell membranes and other cell structure containing lipids.

IV. Carotenoids (Lipochromes)

These are red or orange cell pigments, soluble in organic solvents, but insoluble in water. This group includes the *Carotenes* in carrot and grass, *Xanthophyll* in leaves, vitamin, A egg yolk pigment. These are synthesized by the mitochondria of the cell.

Nucleotides and Nucleic Acid

Nucleotides are involved in at least two major cellular functions:

1. They are monomeric units from which DNA and RNA polymers are constructed, and
2. They act as agents in certain energy-transferring reactions during metabolism.

A mononucleotide is made up of one nitrogen-containing organic base, onepentose sugar, and one phosphate residue derive fromphosporic acid. When there is no phosphate group, the sugar-base combination is called a *nucleoside* (Table 1.3). For this reason, *nucleotides* (phosphate-sugar-base) are also called nucleoside phosphates. Nucleoside mono-, di-, and tri-phosphates contain one, two or three phosphate groups respectively.

The nitrogenous bases commonly found in nucleic acids and their nucleotide building blocks are derivatives of purine and pyrimidine. The commonly occuring purines *adenine* and *guanine* are found in both DNA and RNA, as in the pyrimidine compound *cytosine*, the second kind of pyrimidine in DNA is *thymine*, while its demethylated form, *uracil*, occurs in RNA.

Since each kind of nucleic acid contains one unique pyrimidine, it is convenient to study synthesis and activity of DNA or RNA using isotopically-labelled precursors containing one or the other of these bases. Usually the nucleosides uridine or thymidine, or their nucleotide forms, are added to the biological system under study.

The only difference in the pentose sugars of nucleotides is the presence of a hydroxyl group at carbon atom 2 of D-ribose in RNA, but a hydrogen at carbon-2 of 2-deoxy-D-ribose in

TABLE 1.3
Showing Nomenclature of Nucleic Acids and Their Constituent Units

Base	*Nucleoside*	*Nucleotide*	*Nucleic Acid*
Purines:			
Adenine (A)	Adenosine	Adenylic acid	RNA
	Deoxyadenosine	Deoxyadenylic acid	DNA
Guanine (G)	Guanosine	Guanylic acid	RNA
	Deoxyguanosine	Deoxyadenylic acid	DNA
Pyrimidines:			
Cytosine (C)	Cytidine	Cytidylic acid	RNA
	Deoxycytidine	Deoxyadenylic acid	DNA
Thymine (T)	Thymidine	Thymidylic acid	DNA
Uracil (V)	Uridine	Uridylic acid	RNA

DNA monomers and polymers. This seemingly simple difference is partly responsible for profound differences in stabilities, pairing potential, and functions of DNA and RNA.

Polynucleotides of both DNA and RNA varieties are built from mononucleotides that are linked covalently via phosphodiester bridges between the 3' position of one unit and the 5' position of the next. Since there is no restriction on the vertical sequence of adjacent mononucleotides in either DNA or RNA, a considerable variety of molecules is possible even though only 4 kinds of nucleotide monomers (one for each of the four kinds of bases in the combination with sugar and phosphate) are used in polymer construction. The theoretical variety is calculated as 4", where 4 is the number of different kinds of nucleotides, and n is the number of monomers in the polymer. For a molecule made of only 75 monomeric units, as in some of the smallest RNAs, there may be 4^{74} different arrangements of the constituent units. Each arrangement theoretically constitutes a molecule of different specificity. Where the average gene may include about 500 nucleotides in a DNA sequence, 4^{500} different sequences are theoretically possible and, therefore, that many different and specific genes. Despite the apparently meager number of monomer types an astronomically high number of possible genes can be constructed.

Such variety can easily account for all past and present life forms.

The DNA Double Helix: DNA molecules usually have regular helical configurations because most DNA molecules consist of two *complementary polynucleotide strands*. Fig. 1.6. The two strands are held together by *hydrogen bonds* between complementary pairs of purines and pyrimidines. Adenine always binds with thymine while guanine always binds with

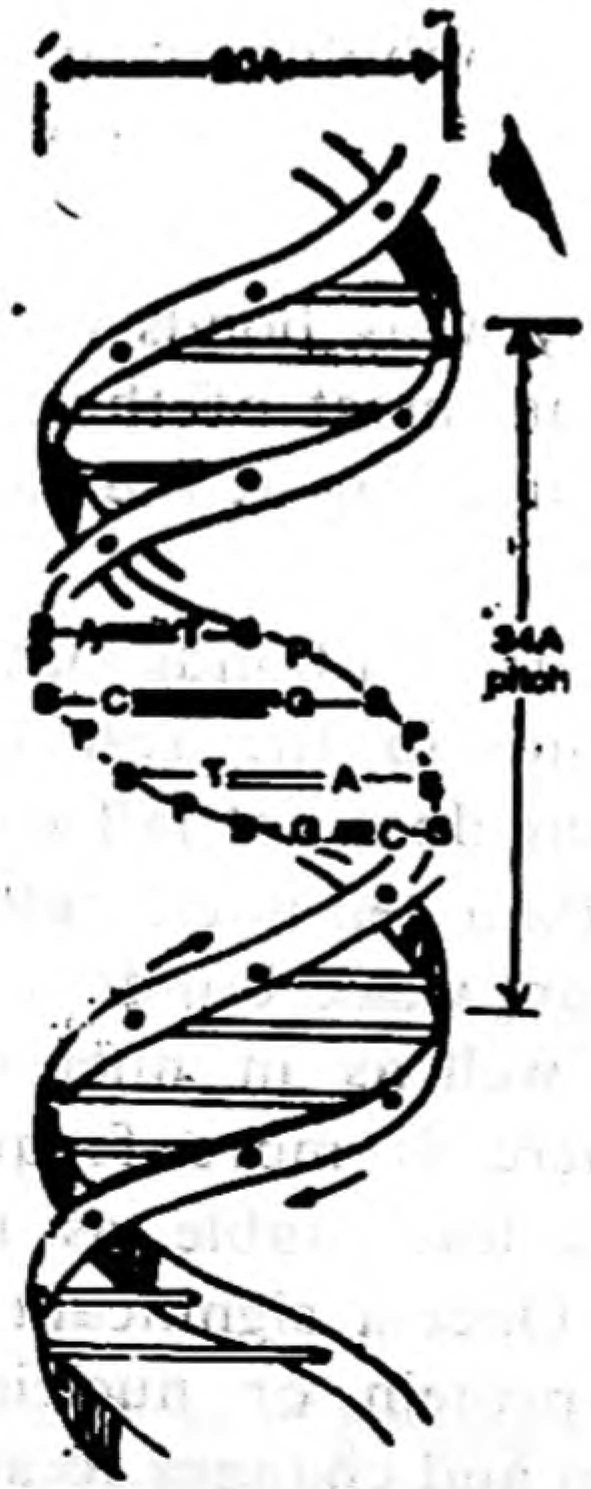


FIG. 1.6: DNA and its paired bases.

cytosine. This repeated hydrogen bonding within the double helix structure and bonding between virtually all the surface atoms in the sugar and phosphate groups with water molecules serve to stabilize the structure.

Since the purine-pyrimidine pairs are found in the center of the molecule, their flat surfaces can stack on top of each other and thereby limit their contact with water. In double-helical molecules, a regular structure is possible because the complementary base pairs are exactly the same size. Single polyuncleotide chains could not have a regular backbone structure because pyrimidines are smaller than purines, which would cause the angle of helical rotation to vary with the sequence of bases.

DNA double helix molecules are very stable at physiological temperatures because:

1. disruption of the double helix breaks hydrogen bounds and brings hydrophobic purines and pyrimidines into contact with water, which is energetically unsatisfactory; and
2. there are many weak bonds within the DNA molecule, arranged so that most of them cannot break without many others breaking at the same time.

Even though some hydrogen bonds may be broken by thermal motion, hydrogen bonds in the rest of the molecule remain intact and the molecule does not fall apart. In fact, when held together by more than ten nucleotide pairs the double helices are quite stable at room weak bonds is stability of molecular shape, in proteins as well as in nucleic acids. At abnormally high temperatures there is more frequent breakage of weak bond, which becomes less stable as temperatures rise above physiological levels. Once a significant number of weak bonds have been broken, a protein or nucleic acid molecule usually loses its original form and changes to an inctive or denatured form.

The Nucleus

The *nucleus* of the meristematic cell may be observed in the resting condition or in any of the various stages of nuclear division. Recent work on nuclear structure appears to show that two tppes of resting nucleus occur in different plants. One type is known as the "solid" nucleus, the other as the "prochromosomal" or vesicular type. It must be borne in mind, however, that detailed study of nuclear structure is only possible, so, far, as the result of first "fixing," then sectioning the meristematic tissue. The sections have to be "stained" with certain dyes. Technique, as well as treatment, plays an important role in this process: choice of fixative and of stain, amount of staining and, above all, resolving power of the microscope at high magnifications are all fundamental to successful interpretation of what is observed. Moreover, certain observed phenomena may be the result of treatment, and recorded observations must make all due allowance for these.

The prochromosomal type of nucleus (Fig. 1.7) consists of a vesicle of sap around the periphery of which is the whole of

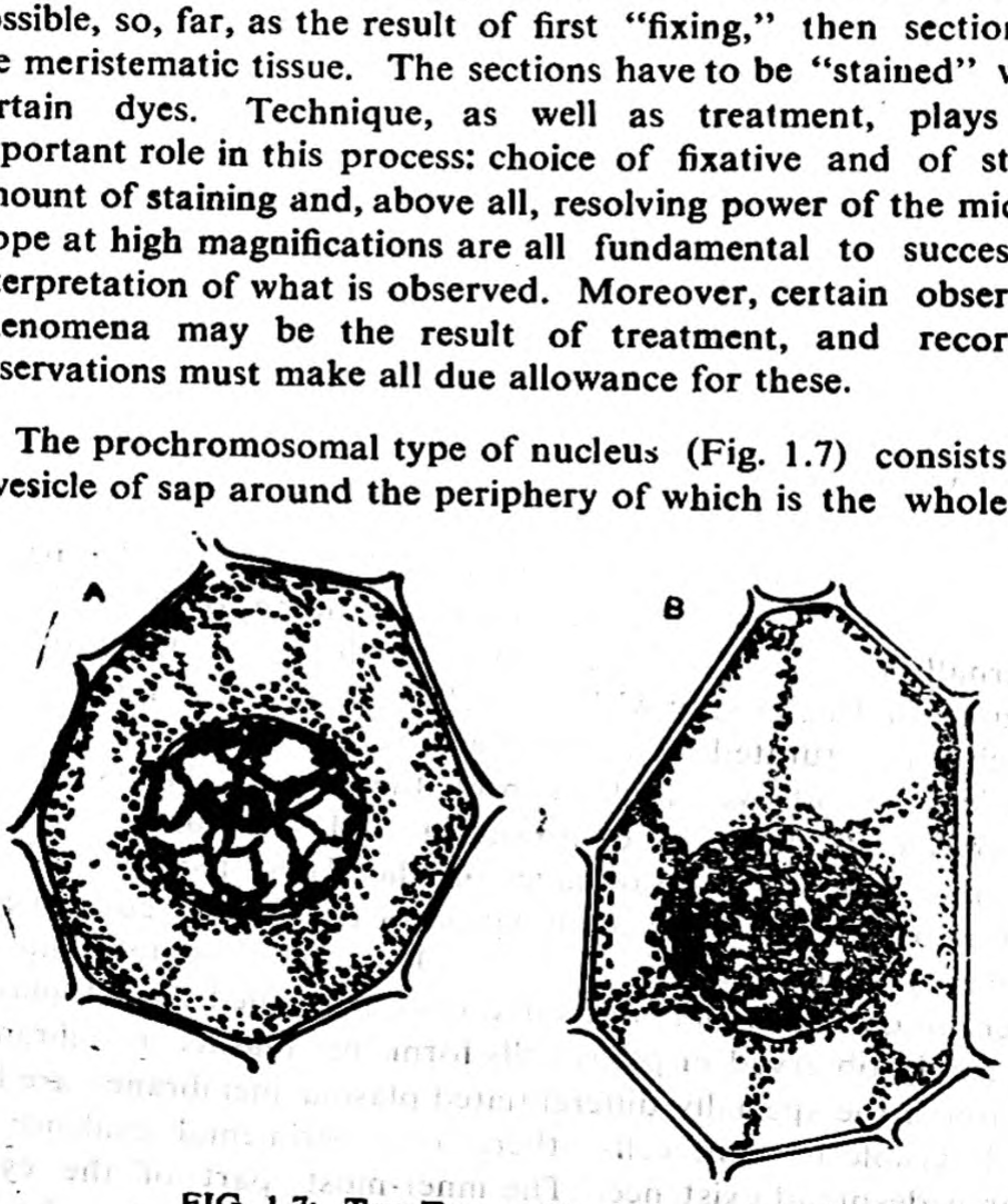


FIG. 1.7: Two Types of Resting Nucleus:
A. Prochromosomal
B. Solid.

the stainable material of the nucleus, the chromatin, included in relatively small bodies called prochromosomes. One or more nucleoli are also present.

The "solid" type of resting nucleus (Fig. 1.4 B) is devoid of free sap and, after fixation, has the appearance of a uniform meshwork of chromatin filling the whole nucleus except for the space occupied by one or more nucleoli. The latter, at least in the early stages, are fluid. This type of nucleus is found in members of the Liliaceae, in *Vicia* and *Osmunda* (the royal-fern). The chromatin meshwork is frequently referred to as a "reticulum", composed of "karyotin."

"The nucleus plays an important part in governing the metabolic processes which go on within the cell, resulting in cell-division, growth and differentiation. The nucleus also contains the hereditary characters which are passed from parent to offspring.

The Cytoplasm

In mature living cells the *cytoplasm* (that part of the protoplasm distinct from the nucleus) forms a lining to the cell-wall. Internally the cytoplasm is in contact with the contents of the vacuole, and externally with the cell-wall and the liquid with which it is saturated. The cytoplasm is not homogeneous. On physical grounds we should expect the surface layers of the cytoplasm to differ in composition and structure from the innermost parts, a knowledge of the laws of surface tension leading us to expect an accumulation of the fat-like components of the cytoplasm at the surface. The differentiated limiting layers frequently visible in animal cells and called the ectoplasm, but rarely observed in plant cells form the plasma membranes. Although the specially differentiated plasma membranes are but rarely visible in plant cells, there is experimental evidence of their widespread existence. The inner-most part of the cytoplasm (endoplasm) contains specialised protoplasmic bodies and non-living inclusions.

The most conspicuous protoplasmic bodies are the plastids. In young meristematic cells they are minute, and have been called proplastids. They multiply by direct division, either at this stage or at cell maturity. When a cell divides, proplastids are present in the cytoplasm of each daughter cell, and as the daughter cell grows the proplastids mature. Mature plastids are classified somewhat arbitrarily according to their colour into two chief types; chromoplasts, which are coloured, and leucoplasts which are colourless.

Ribosomes. These are tiny, darkly staining particles present either attached to ER or freely distributed in cytoplasm. Ribosomes were first discovered in 1941 by Claude, who called them *microsomes*, Palade (1956) named them ribosomes. Usually the membrane bound ribosomes (membrane piece + ribosomes) are called the microsomes. Ribosomes are very significant as the sites of protein synthesis. They are called engines used by cell in protein synthesis. In cells which are highly active in protein synthesis, simple ribosomes get transformed into clusters called *polyribosomes.*

The number and concentration of ribosomes are related to the metabolic state of the cell.

Measurement of the sedimentation coefficient by ultra-centifugation has revealed the presence of two sizes in ribosomes. In bacteria they have a coefficient of 70s whereas in cukaryotic cells they have about 80s.

Ribosomes are generally spheroidal and have a size of 250Å × 150Å. Each spheroid has two sub-units, a larger (140—160Å) and a smaller (50—60Å) one. The smaller one is attached in the form of a cap to the larger sub-unit.

Chemical analysis of ribosomes reveals the presence of RNA and proteins.

Lysosomes

Discovered in 1955 by de Duve, these are the cytoplasmic organelles containing enzymes that bring about *lysis* (breakdown). These are more abundant in animal cells that in point cells. Popularly these are called '*suicide bags*', since they bring about the breakdown of the cell. In shape, lysomes are highly variable. In size, they measure from 0.25—0.8 μ Lysosomes are enclosed in a single layered membrane which is made up of lipoproteins. Inside the membrane is present a homogenous dense granular matrix. The matrix is composed of the lytic enzymes, *e.g.*, acid phosphatase, ribonuclease, β galactosidase etc.

Various theories have been put forward to account for the origin of lysosomes. The most important theories are:

1. They develop from extracellular pinocytic vesicles;
2. They develop as cytoplasmic inclusions containing digested material.

Recent evidences seem to point out that lysosomes may be derived from the golgi complex.

Centrosome (centriole). Centrosomes are usually present close to the nucleus and are two in number. They are intimately associated with the cell division. Their presence in the plant cell is rather doubtful.

Plastids

The *plastids* are differentiated bits of protoplasm—"organs," or areas, of metabolic activity associated with particular functions. They have a limiting, apparently semipermeable membrane and complex internal structure. They are often coloured and usually conspicuous. In size they are small, and generally many occur in a cell (Figs. 1.7-A 1.9). They are variable in shape but rounded types are most common. Spherical ovoid, diskoid, granular, rod-like plastids all occur frequently. Large plastids of peculiar shape are present in many of the algae and rarely elsewhere. They may be found in a living cells of a plant and probably are present in every cell in its early stages of development. Later they become resricted to certain cells and are abundant only in those which have specialized functions, such as photosynthesis, storage, and colour manifestation.

Origin. Plastids are present in large numbers in young meristematic cells (Fig. 1.7A) where they are minute, the smallest being at the limit of microscopic visibility. At this stage, when they are known as *proplastids*, they are rounded bodies that do not resemble plastids. As the cell grows, proplastids mulitply freely and mature plastids gradually develop. Increase in number by division continues at all stages but less frequently in mature plastids. Probably plastids arise only from preexisting plastids.

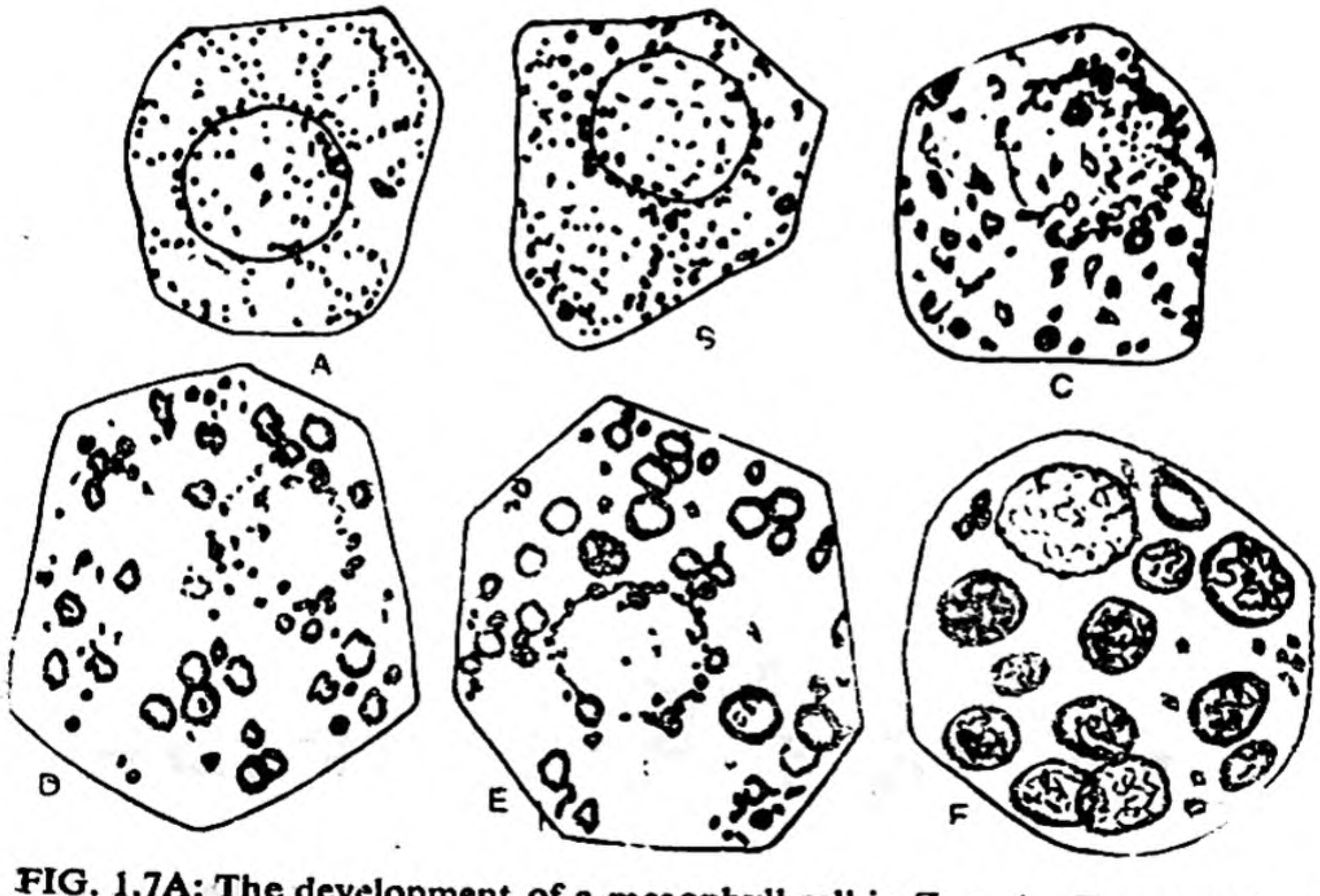


FIG. 1.7A: The development of a mesophyll cell in Zea. A—F, successive stages: the nucleus decreases in proportionate size; vacuoles appear, enlarge, and fuse; chloroplasts develop from proplastids. (After Randolph.)

Types of Plastids. Several fairly distinct types of plastids occur, but all are of similar fundamental nature. They fall into two chief classes: coloured plastids, *chromoplasts*, and colourless plastids, *leucoplasts*. Green, chlorphyll-bearing chromoplasts are known as *chloroplasts* and are commonly set apart as a third major type because of their important function in food manufacture. This leaves the term "chromoplast" to cover all coloured plastids except chloroplasts, and this is its general use.

Chloroplasts. The chloroplasts of the higher plants are mostly uniform in size and chiefly flattened-ellipsoid or disk-like in shape. They are numerous—from a few to very many in a cell—and small, averaging 5 micra in diameter. They multiply by constriction, and may at other times change shape, appearing as though semiliquid. The chlorophyll is wholly or largely restricted to numerous small granules known as *grana* (Fig. 1.8). Apparently these deeply coloured bodies are commonly so arranged and so closely packed that the plastid appears structurally homogeneous. Sometimes an obscure layering can be seen (Fig. 1.8,C). Chloroplasts of shade leaves are somewhat larger than those of leaves in full light on the same plant and their chlorophyll content is possibly greater per unit volume.

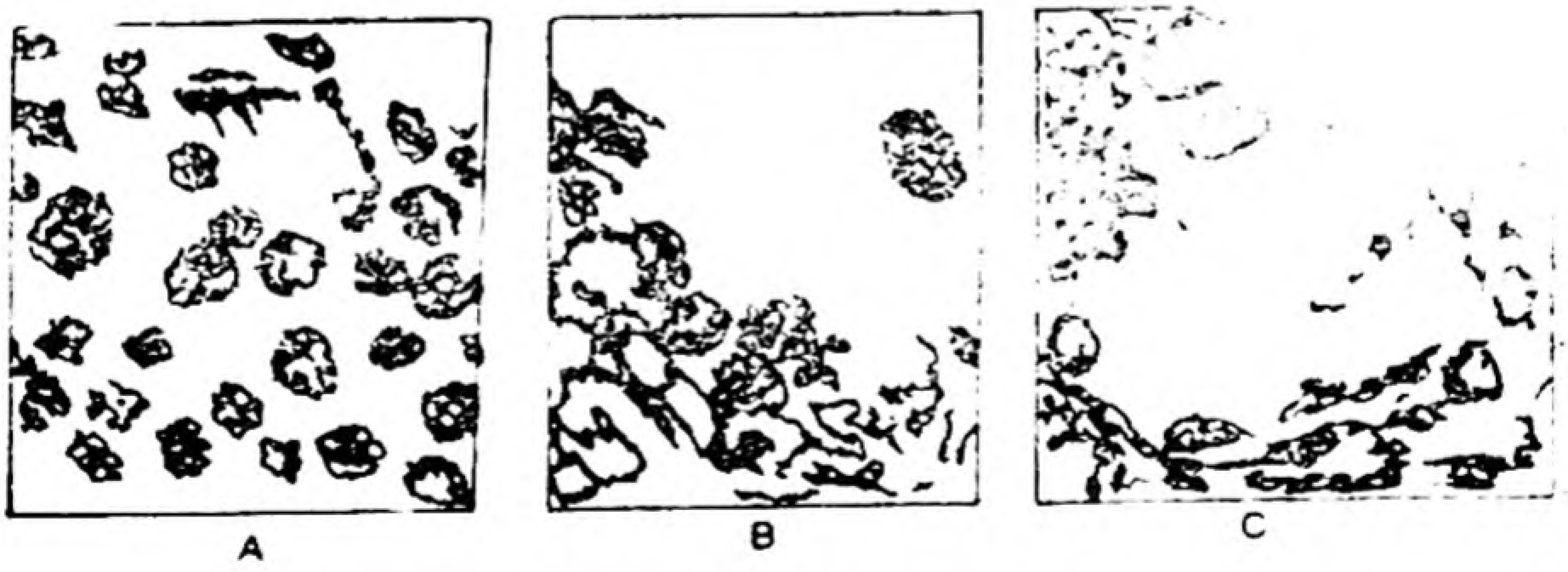


FIG. 1.8: Minute structure of chloroplasts. A, Aponogeton; B.C. Todea superba. A, B, grana large and free; C, grana small and aggregated in plate-like layers. (After Heitz.)

Chromoplasts. Nongreen chromoplasts range in colour through yellow, orange, and yellow-red. The colour is given chiefly by xanthophyll, carotin, and carotinoids. Chromoplasts show great variety in shape but are chiefly irregular; granular, angular, acicular, and forked types occur (Fig. 1.9). The irregular and sharp-pointed shapes are believed to be caused in part by the presence of the coloured substances, especially carotin and carotinoids, in crystalline form, as in the root of *Daucus* (Fig. 1.9A). The functions of these plastids are obscure. They are associated with colour in flowers and fruits but occur also in other regions such as roots. Chromoplasts commonly represent transformed chloroplasts, but may form directly from small leucoplasts.

Leucoplasts. The term "leucoplasts" covers various types of colourless plastids. The early stages of all plastids are called leucoplasts but such young plastids are best called *proplastids*.

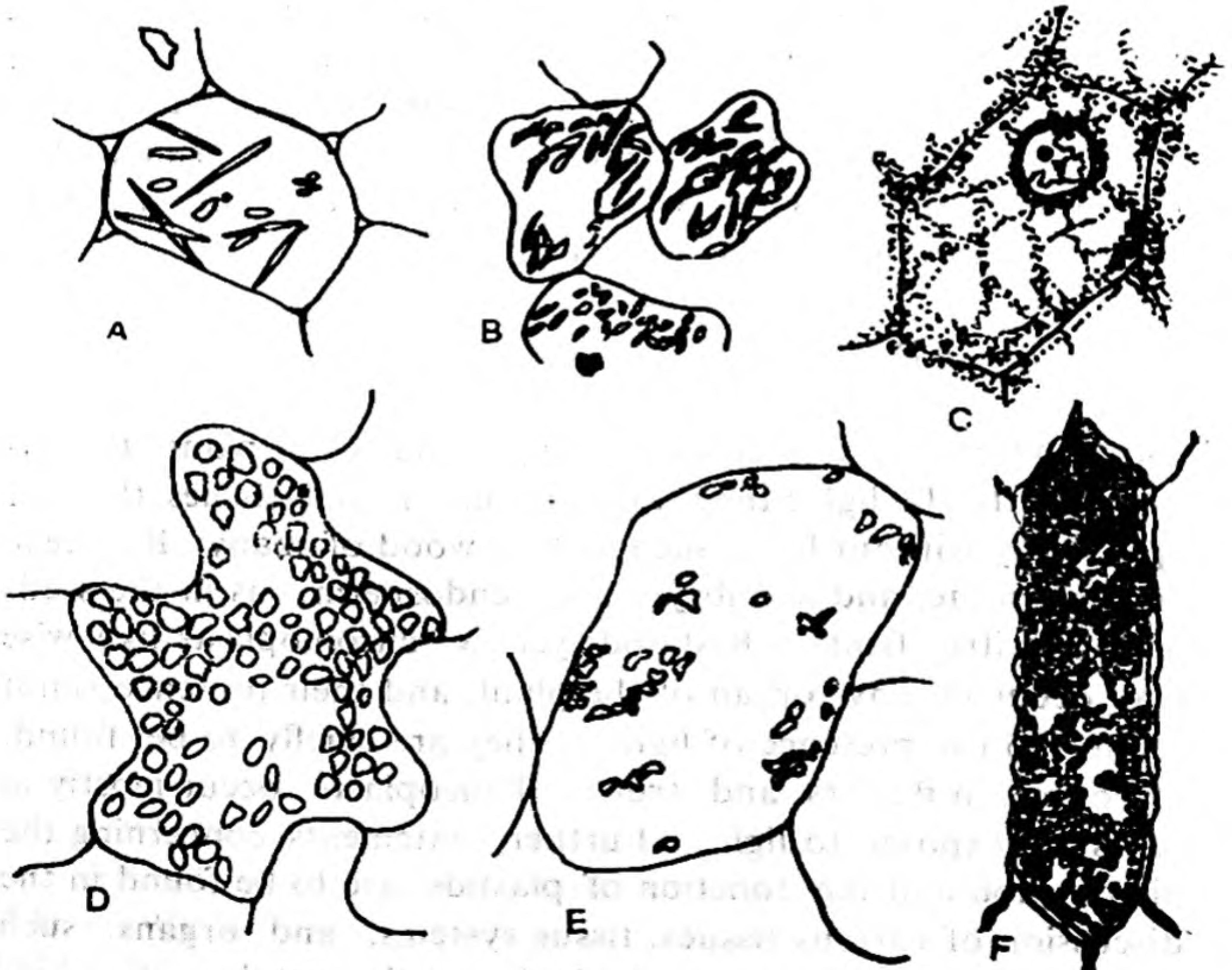


FIG. 1.9: **Plastids, Chromoplasts: A, in cortex cell of root of Daucus; B, in pulp cells of fruits of Arisaema; D, in cell of petal of Forsythia: E, in pulp cells of fruit of Lycopersicon; F, in cell of corolla of Taraxacum. Leucoplasts: C, in young endosperm cells of Zea.**

and only mature colourless plastids should be called leucoplasts. Like chromoplasts, leucoplasts, vary in shape; extreme forms are rod-like. They change shape readily and are probably always highly plastic. They are concerned with food storage and doubtless have other unknown functions. The type of leucoplast associated with starch-grain formation in storage regions is known as *amyloplast*, Leucoplasts related to the formation and storage of oils and fatty substances are known as *elaioplasts*. These are at least in part amyloplasts that vary in function at certain times. The leucoplasts of hairs and of other epidermal cells are probably degenerate or dormant plastids of other types.

That all plastids are alike in nature is clear from the readiness with which one type is tranformed into another. For example, the chloroplasts of young fruits and of developing petals may become the chromoplasts of the ripe fruit and of the mature flower respectively; the leucoplasts of a potato tuber become chloroplasts on exposure to light.

Occurrence of Plastid Types

Chloroplasts may occur in any part of a plant that is exposded to the light; they also occur in some tissues that are apparently without light, such as the wood of many Rosaceae and Erieaceae, and in enbryos and endosperm, as in the seeds of some citrus fruits. Red and yellow chromoplasts like wise may occur in any organ of the plant, and their presence is not related to the presence of light. They are chiefly to be found, of course, in flowers and fruits. Leucoplasts occur mostiy in parts not exposed to light. Further statements concerning the distribution and the function of plastids are to be found in the discussion of various tissues, tissue systems, and organs, such as collenchyma, the cortex, the leaf, and the petal.

Many protoplasmic bodies smaller than plastids occur in cytoplasm; these are known as *chondriosomes* and *mitochondria*. Some of those known as chondriosomes are doubtless proplastids. The nature and distribution of these smaller cytoplasmic structures lie outside the field of the present treatment.

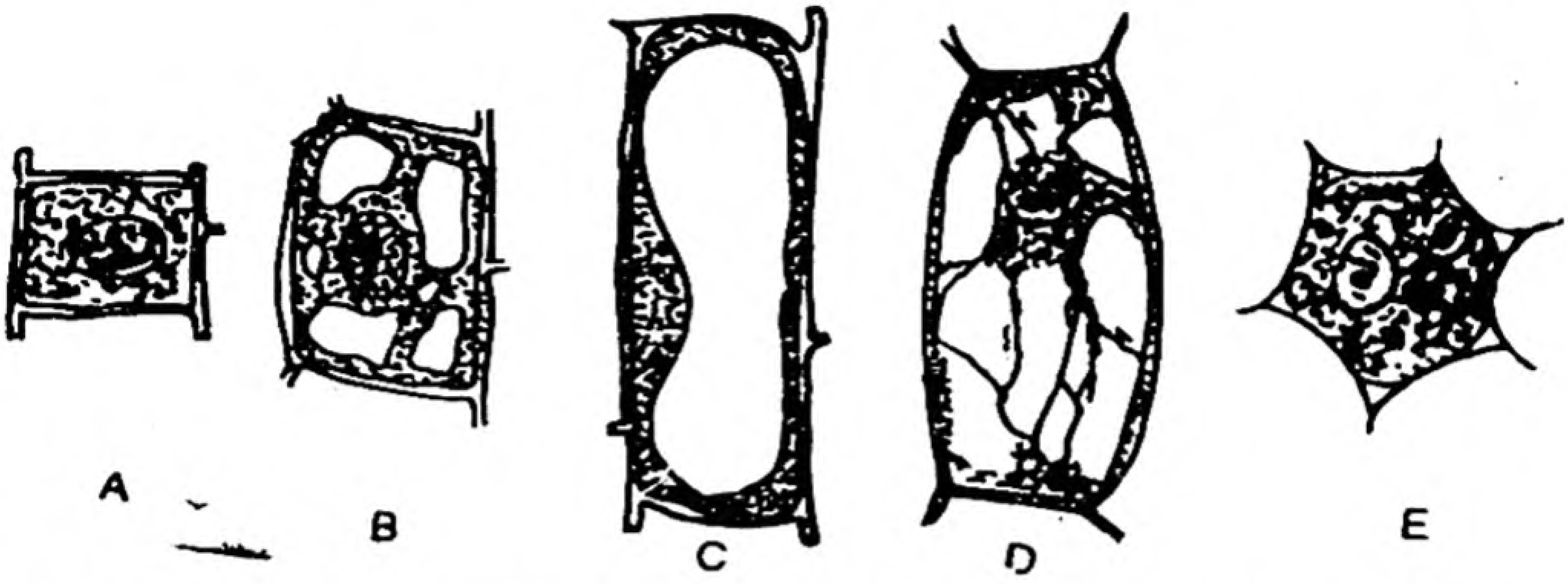


FIG. 1.10: A—C, diagram of a plant cell in three successive stages of development: the vacuoles increase in volume and fuse and the cytoplasm becomes limited to the parictal region. D, cell of stamen hair of Tradescantia, indicating direction of streaming movements in the cytoplasmic strands. E, parenchyma cell from cortex of Polygonella, showing nucleus, plastids, and scanty cytoplasm. (After Sharp.)

Vacuoles and Cell Sap

The cytoplasm of mature and of many young ceds contains one or more cavities known as *vacuoles*. Conspicuous cavities usually develop as the cell matures, beginning as one or several small cavities and enlarging and fusing until in the mature cell there is usually one large central vacuole which restricts the cytoplasm to a thin layer lining the wall (Figs. 1.10C). The nucleus, plastids, and most inclusions are contained in this peripheral layer. Frequently strands of the cytoplasm extend from the outer layer as irregularly anastomosing branches through the central vacuole; under these conditions the nucleus may occupy a median position in the cell. The vacuoles and their liquid content, the *cell sap*, constitute the cell *vacuome*.

The cell sap is a watery, nonprotoplasmic liquid consisting of water with various substances in solution—inorganic salts, carbohydrates, proteins, amides, alkaloids, pigments, etc. These substances may be mineral nutrients, elaborated food, waste products, or substances of unknown relation to metabolism. In the physiology of the cell the significance of these substances dissolved in the cell sap is not well understood.

Colour in Cells

Substances that give colour to cell are found chiefly in the plastids and the cell sap. The cell wall, cytoplasm, and nucleus are commonly nearly colourless. The green colouring substance of chloroplasts is *chlotophyll*, in two forms known as *a* and *b*. With the chlorophyll, but masked by it, are yellow pigments, *earotinoids*. Included in the carotinoids are carotins, carotenes, and xanthophylls. These substances give the yellow colour of "golden" varieties of plants (where the chlorophyll content is low) and of leaves variegated with yellow. Leaves variegated with white have little or no colour in the plastids of the pale areas. Abundant carotinoids (sometimes in crystalline form) in chromoplasts give yellow, orange, and yellow-red colours to flowers and fruits. Another group of pigments the *flavones*, are water-soluble and colour the cell sap. In some genera, for example, *Verbascum*, they give yellow colour to corollas. The *anthocyanins*, oxidation products of flavoness, also water-soluble, colour cell sap and give red, purple, and blue colour to many flowers and fruits. A reddish, anthocyanin-coloured cell sap, together with the green colour of the chloroplasts, gives the bronze or purple colour to the leaves of purple-leaved forms of many plants. Anthocyanins are also present in young twigs and leaves, especially if growth is taking place at low temperatures. Other colour-bearing substances may be present, and lower groups of plants—algae and bacteria—possess some distinctly different substances. In white floral parts there is no colour, and light is reflected from the many semitranparent cells separated by intercellular spaces.

Autumn colour. In slowly dying leaves chlorophyll breaks down into colourless substances. Green leaves become yellow as the xanthophyll is thus unmasked. Xanthophyll in the disorganizing chloroplasts in this way gives much of the yellow colour seen in autumn foliage. Reds and purples are cell-sap colours, resulting from oxidation of flavones, and are especially brilliant when formed in the presence of sugars and abundant sunshine. These cell-sap colours, in combination with some persisting chlorophyll, xanthophyll, browning cell walls, tannins, and less common colour-giving substances, give great variety in autumn colour. The changes from green foliage are not caused by frost, although frost may hasten the process. Many plants whose leaves colour brilliantly in cool temperate climates show equally bright colour when grown in frostless climates. Dying branches of tress often slow full 'autumn' colour in midsummer.

Vacuoles

The cytoplasm of meristematic cells contains non-living inclusions. Some of these are in the form of minute droplets. Each droplet is a small vacuole filled with cell-sap. These small vacuoles enlarge and finally coalesce, so that ultimately one large vacuole is formed. The enlargement of the vacuoles is accompanied by the entry of water into the cell Fig. 1.11. Therefore it will be evident that with the entry of water into the cell, the size of the cell will increase, and, in fact, the cell attains a volume many times greater than the volume of the original meristematic cell. During this process of cell enlargement by vacuolation the nucleus and cytoplasm do not greatly increase in size, and in the fully vacuolated cell the cytoplasm

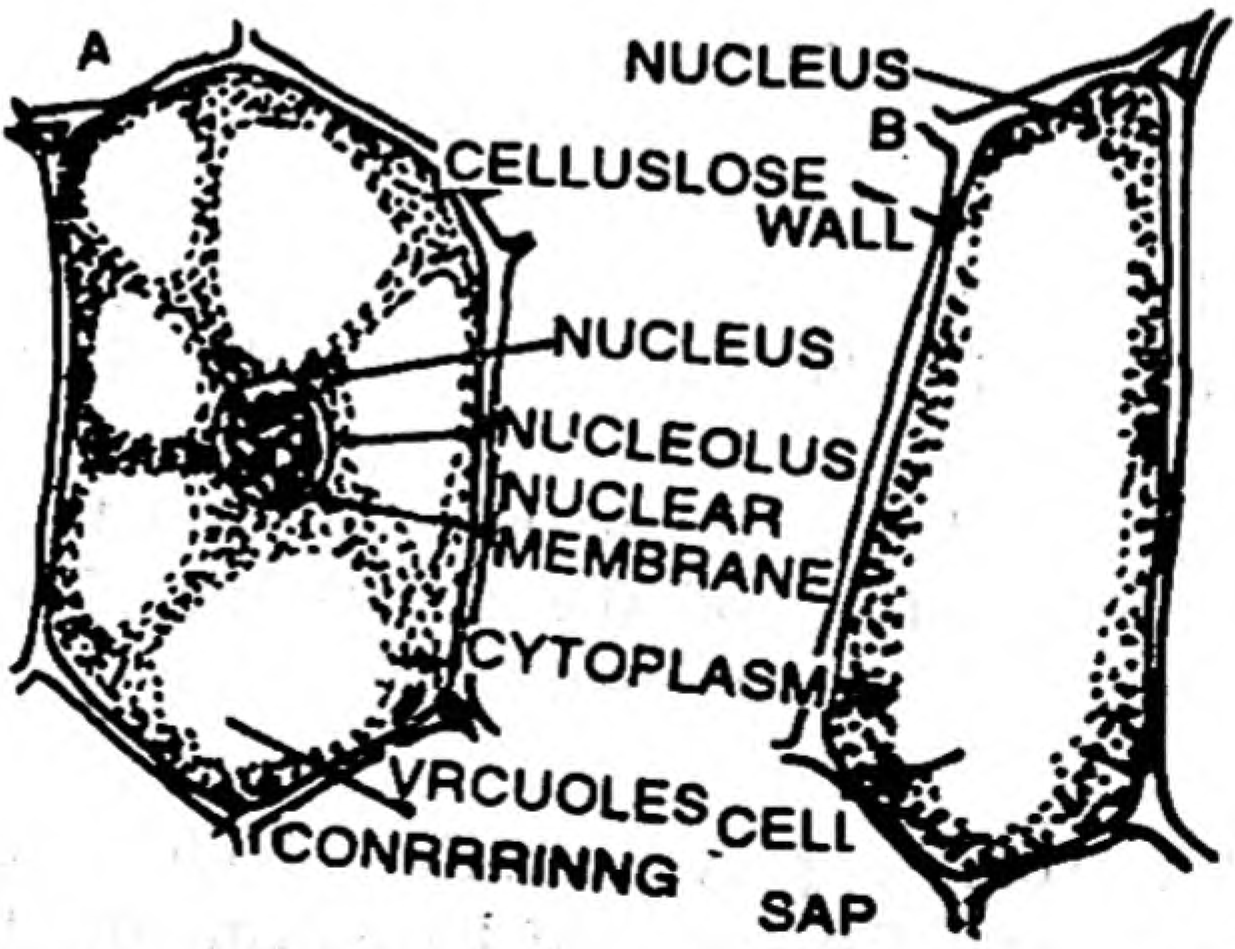


FIG. 1.11: Types of vacuolated cell from fern rhizome.
A. "Suspended" nucleus, B. Peripaeral nucleu.

forms a parietal layer which lines the cell-wall internally, and a number of strands which extend from this parietal layer across the central vacuole. The nucleus may be suspended in the centre of the vacuole by these cytoplasmic strands, or it may be embedded in the parietal layer of cytoplasm. This layer of cytoplasm is sometimes referred to as the primordial utricle, and besides the nucleus it contains plastids, and often various non-living inclusions as well. When cell enlargement is proceeding, the cell-wall is tightly stretched and becomes increased in area, whilst its thickness is maintained or even increased by the deposition of cellulose (generally), formed by the activity of the living protoplasm.

The cell-sap which fills the vacuole consists of an aqueous solution of various inorganic and organic compounds. Inorganic ions are always present, especially those resulting from the dissociation of the nitrates, sulphates and phosphates of certain alkaline and alkaline-earth metals, and carbon dioxide in solution is also present.

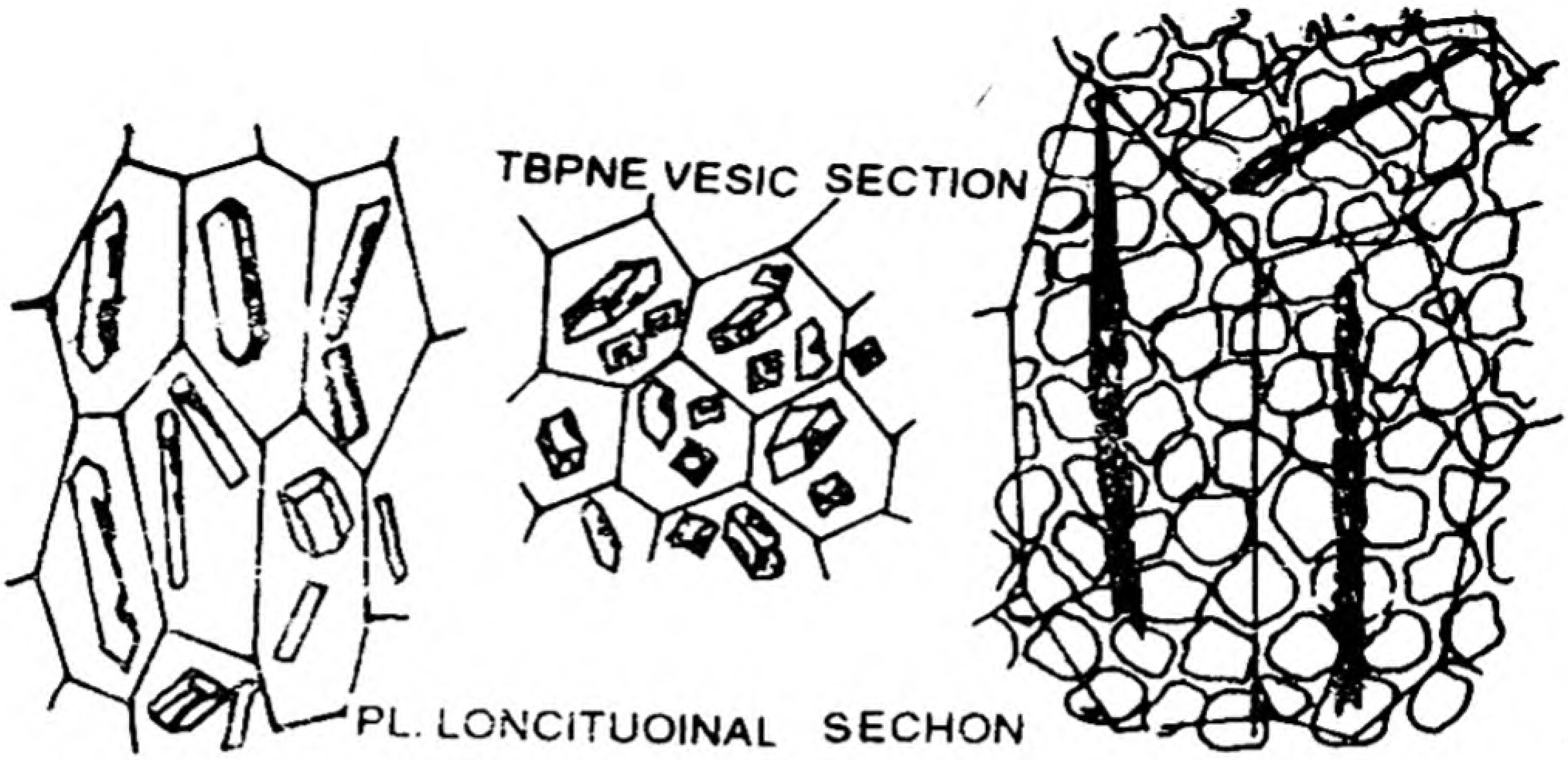
A number of sugars have been isolated from the cell-sap of various plants. Glucose (dextrose) and fructose (laevulose), monosaccharides with the empirical formula $C_6H_{12}O_6$, are especially common, small amounts of glucose being found in most leaves which are exposed to light, and larger amounts in many fruits. These sugars readily reduce Fehling's solution and ammoniacal silver nitrate, and they share this reducing property with a disaccharide, maltose ($C_{12}H_{22}O_{11}$), which is sometimes present in the sap of leaf cells. Sucrose, or cane sugar ($C_{12}H_{22}O_{11}$), is a non-reducing disaccharide widely distributed in plants, being abundant notably in the root of the sugar-beet and the stem of the sugar-cane. These form the commercial sources of "cane sugar."

A complex, soluble polysaccharide, inulin, occurs in the cell-sap of the tubers of dahlia and Jerusalem artichoke and in the root of the dandelion. Although soluble in water inulin is insoluble in alcohol, and hence when inulin-containing tissues are preserved in alcohol, the inulin is precipitated in the cells in the form of sphaerites (sphere crystals).

Other carbohydrates of less common occurrence in the cell-sap include dextrin, mannitol and various pentosans, whilst soluble pectin, which is believed to be a compound of certain sugars with other substances, sometimes occurs in appreciable amount.

Glycosides are also of common occurrence in the cell-sap. These may be regarded as compounds of glucose, generally with aromatic substances, so that when the glycoside is decomposed, and the aromatic substance set free, an aromatic smell is discernible. An example is amygdalin in the bitter almond. This, when decomposed, gives rise to glucose, benzaldehyde, and hydrocyanic acid, and it is to the benzaldehyde particularly that the characteristic odour and taste of bitter almonds is due. Indeed, artificial almond essence consists mainly or wholly of synthetic benzaldehyde. These glycosides are decomposed by enzymes, and generally the appropriate decomposing enzyme is present in the same tissue, but not necessarily in the same cell, as the glycoside. Thus, in bitter almonds the enzyme emulsin splits up the amygdalin. The glucose set free by the decomposition of the glycoside can, in all probability, be utilised by the plant in its metabolism.

Mucilaginous compounds occur in cell-sap, too, and it is to these compounds that the slimy character of many cell-saps is due. Mucilages are found abundantly in the cell-sap of many bulbs, *e.g.*, onion, bluebell, and in the leaves of many succulent plants, and, as we shall see later, are also formed from cell-walls. Mucilages swell or dissolve in water, but are insoluble in alcohol. They are polysaccharide in nature and, when hydrolysed, may yield sugars which reduce Fehling's solution. Reagents in common use for testing for mucilages are solution of ruthenium red, alkaline solution of corallin, dilute solution of methylene blue and chlor-zine iodide.


TBPNE VESIC SECTION
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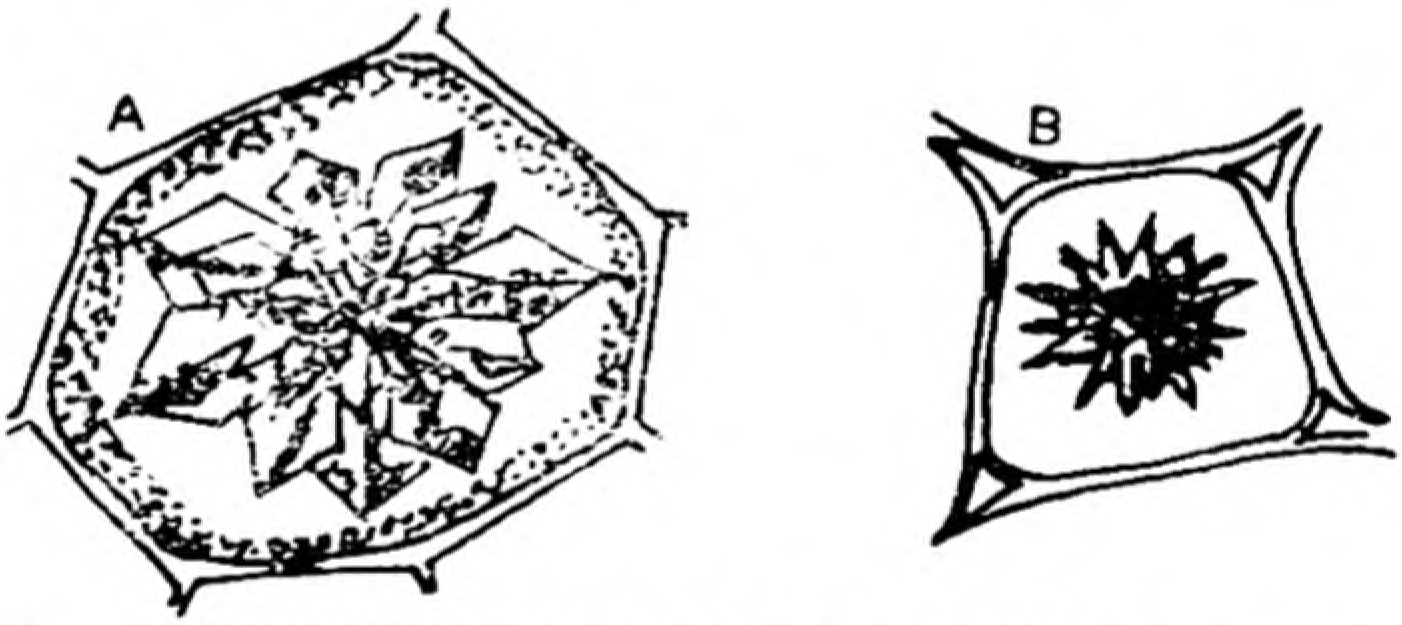
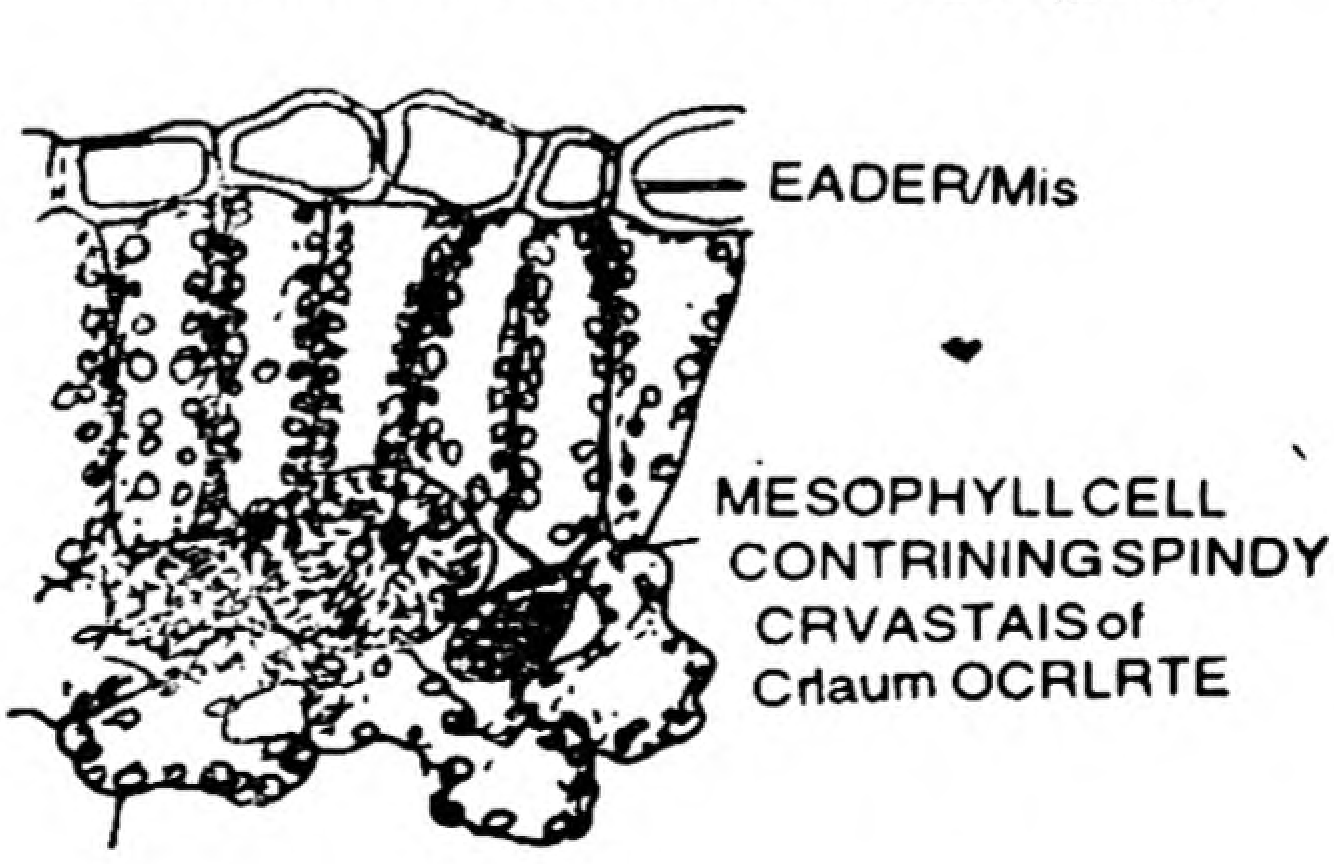
(a) Priamatic cryststs in the phloem of Quillaja Saponaria

(b) Raphides in Leaf-Cells of circaca Lutetiana



(c) A and B Dmesesin Rheum and Begomia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MESOPHYLLCELL CONTRININGSPINDY CRVASTAISof Crlaum OCRLRTE

FIG. 1.12: Types of calcium oxalate crystals.

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Tannins may be present in cell-sap. Treated with ferric salts they give either a blue-black or green colour. They are complex substances, and are classified according to their behaviour when treated with boiling dilute sulphuric acid. They are soluble in water and alcohol and are best examined after extracting, but if it is desired to examine them *in situ*, the plant tissue containing them should be sectioned dry and amounted direct in the reagent, *e.g.*, dihate solution of ferric chloride.

Anthocyanin pigments are frequently present in cell-sap. These pigments as a rule give blue or red (or intermediate) colours to the cell-sap, and are the cause of many floral colorations; whilst a similar type of compound is responsible for the red coloration of the root of the garden beet.

The cell-sap is often acid in reaction, either because of the presence of free organic acids, or their acid salts. Malic, citric and tartaric acids are all of common occurrence, and the acidity of many fruits is due to the presence of these acids in the cell-sap. Oxalic acid, too, is of general occurrence, but it is usually present, not as the free acid, but in the form of its insoluble calcium salt, and as such is usually regarded as a waste product of cell metabolism, and of no further use to the plant, whereas the other organic acids mentioned may be used in respiration or in other ways.

Calcium oxalate occurs in a crystalline form, and the crystals may be of various types Fig. 1.12 and 1.14 the commonest belonging to the monosymmetric (monoclinic) system. These crystals are widely distributed in plant tissues in the form of:

1. Single crystals, *e.g.*, square prisms, double pyramids, rhombohedra, etc. Sometimes these are so large as to fill the cell, distorting the cell-wall to their shape. The cells containing these crystals may be scattered throughout a tissue, as in Quillaia bark or Quassia wood, or they may be arranged as a crystal sheath around bundles of sclerenchymatous fibres as in Cascara bark and Liquorice root, or vascular bundles as in Senna

leaf. Included under single crystals are needle-shaped (acicular) ones named raphides, usually associated together in bundles in enlarged parenchymatous cells containing mucilage. Such bundles of raphides are particularly met with in Monocotyledons.

2. Druses, cluster-or rosette-crystals, which are spheroidal groups of tetragonal crystals, frequently built up around an organic core. Large druses are present in *Rheum*, and minute ones, associated with aleurone grains, in the endosperm cells of fruits of Umbelliferae.
3. Crystal-sand, masses of micro-sphenoidal crystals packed into a cell. Examples are seen in leaves and roots of *Atropa belladonna.*

Crystals of calcium oxalate dissolve in any of the mineral acids without effervescence; they are insoluble in dilute acetic acid, even on warming. Dilute hydrochloric and acetic acids are therefore employed in microchemical tests for calcium oxalate. By this means it is distinguished from calcium carbonate, which dissolves in dilute acetic acid.

Calcium carbonate occurs only infrequently in plants; it is best known in those curious structures called cystoliths, which consist of a peg-like ingrowth of the cell-wall into the cavity of epidermal cells of *Ficus* leaf. In and around this protrusion calcium carbonate is deposited. Cystoliths are found mainly in the members of the families Moracease and Acanthaceae, and may be stalked or sessile.

Soluble nitrogenous compounds may be present in the cell-sap. We have mentioned already that inorganic nitrates may occur. Ammonium compounds may be present too, and also relatively simple organic compounds of the nature of amino acids and amides, and some of these may represent steps in prote synthesis.

Besides the proteins which form a part of the living protoplasm, non-living proteins may occur either dissolved in the cell-sap or in the form of crystal-like bodies, crystalloids. In many seeds, especiylly those containing oil, the vacuoles contain large amounts of dissolved proteins, and as the seed dries out and ripens these proteins may be transformed into *aleurone grains*. Each grain has a membrane, and this encloses a mass of protein in which bodies are embedded. One of these, the larger, is the crystalloid, which consists of protein. The other globoid bodies, which are smaller than the crystalloid, consist of protein combined with phosphates. Treatment with solvents resolves the aleurone grain into its components. Cold water dissolves away the ground matter of the grain, leaving the crystalloid and globoids inside the membrane. Dilute caustic potash slowly dissolves the membrane and the crystalloid, leaving the globoids unaffected.

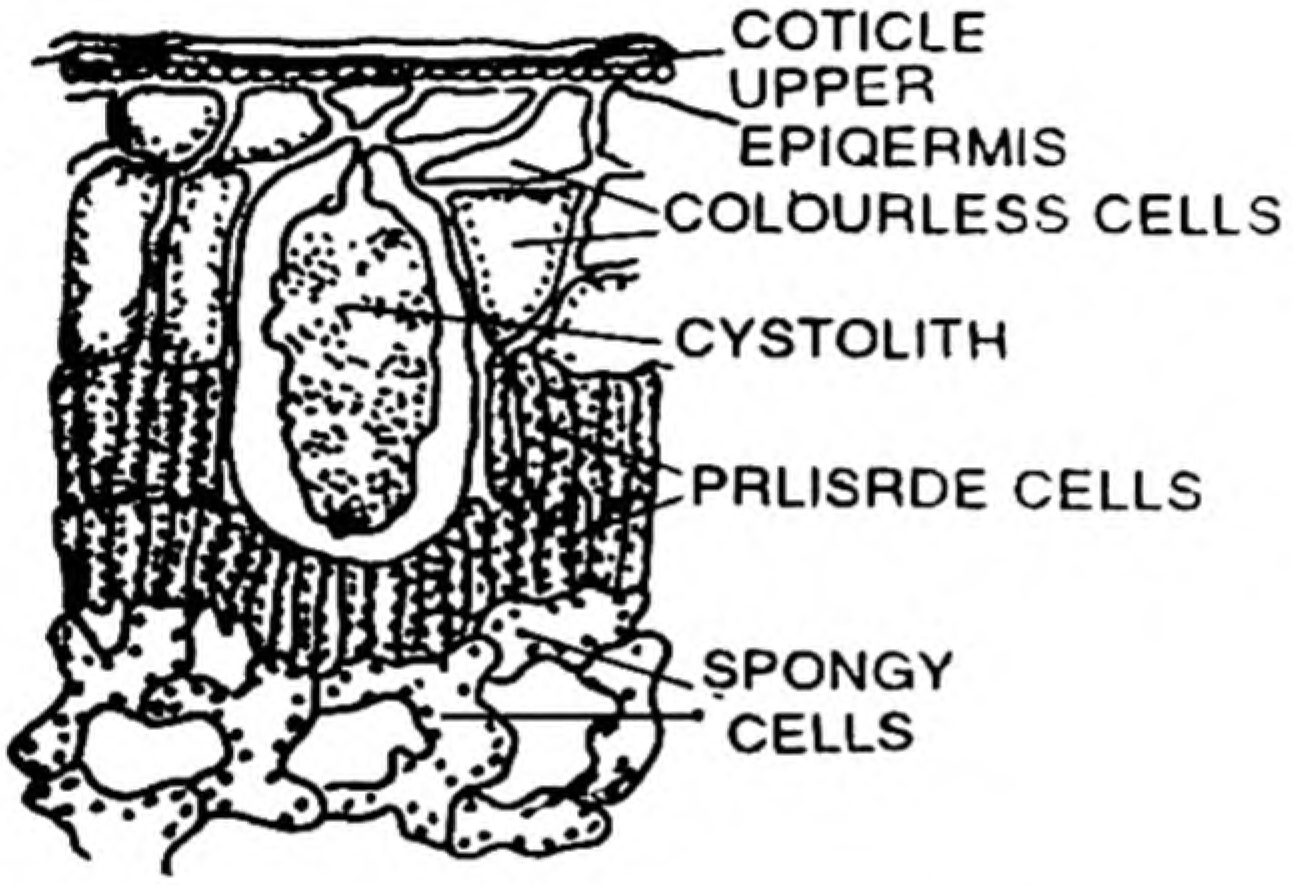


FIG. 1.13: Cystolith in leaf of ficus.

Fats and *oils* are frequently present in plant cells, especially in certain seeds. They occur as droplets or globules, and may be present either in the cell-sap or protoplasm, or both. Fats and oils are compounds of glycerol and various fatty acids, mainly oleic, palmitic and stearic acids, and they often form an important kind of food reserve. Similar chemically, fats and oils differ only in their consistency, fates being solid at ordinary temperatures, and oils liquid. Fats and oils found in plants are generally liquid, but there are a few exceptions. Cocoa butter, from cocoa "bean," and the fat from the seeds of the nutmeg both have high melting points and are solid at normal temperatures. but in the plant they are probably present, not as solid fat particles, but in the form of an emulsion. Microscopically, fat globules may be distinguished by their reactions with certain stains. They stain red with tincture of alkanna, Soudan III, and Scharlach R.

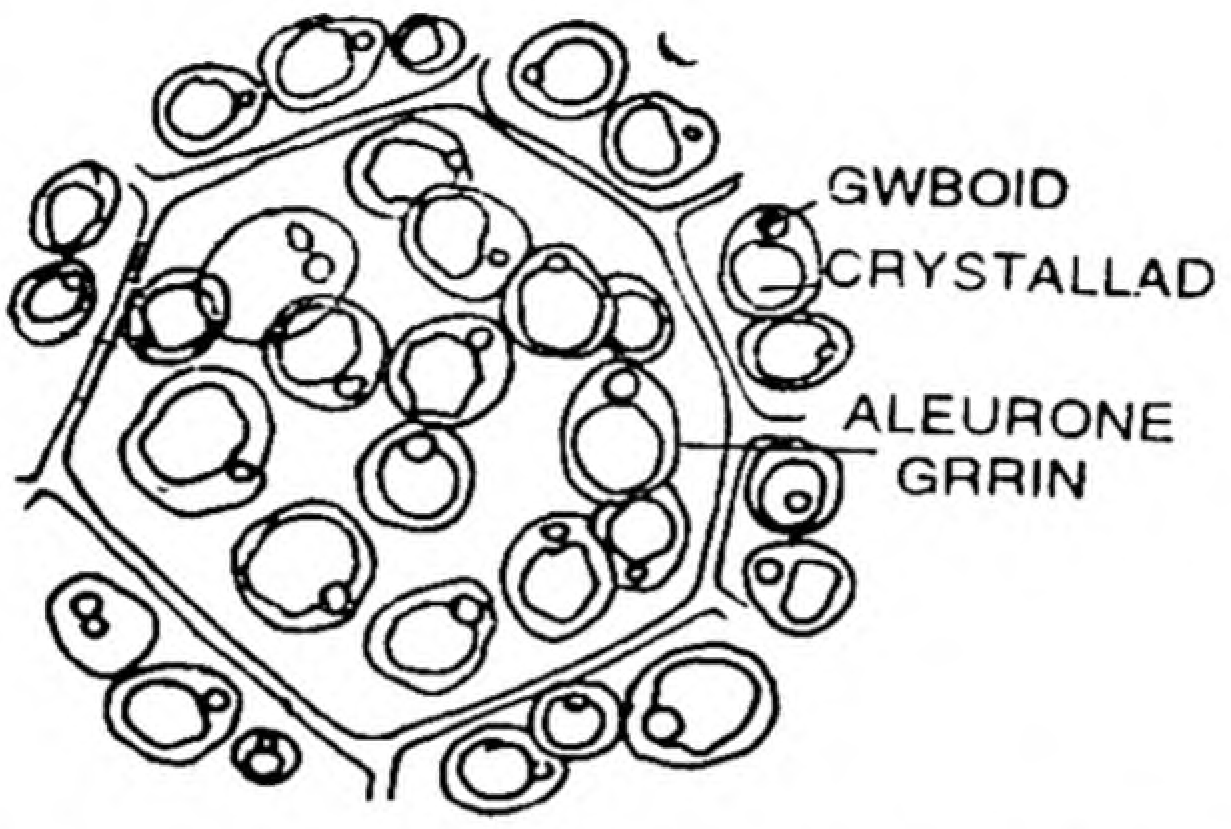


FIG. 1.14: Aleurone grains in endosperm cells of castor-oil seed.

We have by no means exhausted the possible constituents of the cell-sap, but have dealt briefly with the most important ones. Other substances sometimes present include various organic basic substances such as the purine bases and the alkaloids. The functions of these in the plant are not clear, but the alkaloids at least are of considerable therapeutic value.

Resins are found also, but are of limited distribution, being confined to special cells or ducts in certain plants, and odourous ethereal or essential oils are produced by some plants. These essential oils are usually mixtures of complex organic compounds such as alcohols, esters, ketones, aldehydes and hydrocarbons, not all of which are sweet smelling. Essential oil of lemon, for instance, consists mainly of inert hydrocarbons, whilst the smell and taste are due chiefly to a small amount (about 5 per cent) of the aldehyde citral which it contains.

Resin also is not itself a simple chemical substance, but a mixture of substances insoluble in water and containing resin acids, resin esters and resenes, all of which are complex substances of high molecular weight. The relative proportions of these compounds vary in different resins. Colophony contains a high proportion of resin and (abietic acid). Myrrh also is rich in resin acids. Benzoin, Storax, Balsams of Peru and Tolu, and Asafoetida consist mainly of resin esters and free aromatic acids. Sandarac and Mastic contain a considerable proportion of resenes.

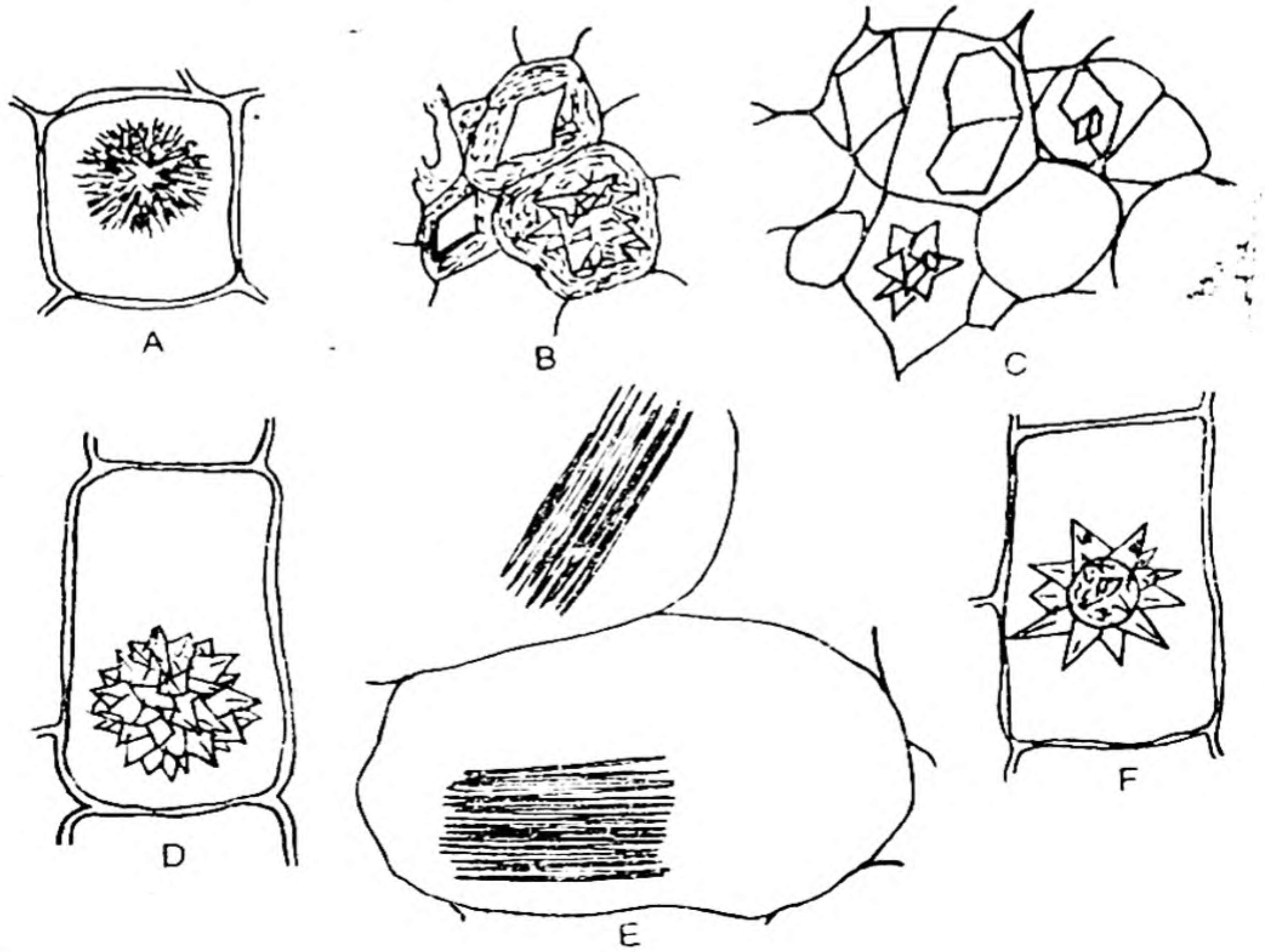
ERGASTIC SUBSTANCES

Many kinds of solid particles, organic and inorganic, as well as such substances as oils, gums, resins, etc., are frequently present within the protoplast, either in the cytoplasm or in the vacuole. These represent, like the dissolved substances, food products, such as starch and aleurone grains; waste products, such as crystals; and other substances of doubtful or unknown function, such as rubber, mucilages, tannins, latex, alkaloids. Gums, resins, tanins, etc., are often present in the lumina of nonliving cells; for example, in the heartwood of *Sequoia* and mahogany (*Swietenia*), and in cork cells of many trees. Some of these substances, for example, starch, occur in most plants; others are characteristic of certain large or small groups of plants, and are lacking in others.

Crystals. Crystals of various chemical nature occur freely in plant cells—of these, salts of calcium, chiefly calcium oxalate, constitute the majority. Crystals of other salts of calcium and of various other inorganic substances, such as silica and gypsum, occur less frequently; crystals of many organic substances, such as carotin, berberin, and saponin, are frequent. All parts of the plant may contain crystals, though these structures are more abundant in certain regions, as in the pith, cortex, and phloem, than in others. There are many forms of crystals (Fig. 1.15) Of these, solitary, rhombohedral crystals, sheaf-like bundles of long particular crystals known as *raphides* (Fig. 1.15E). and clustered crystals in globose masses called *druses* (Fig. 1.15 ABDF) are most common. The individual crystals of druses may be raphides. Solitary raphides, small prismatic crystals, and minute crystals called *crystal sand* are other frequent types. *Cystoliths*, often considered crystalline bodies, consist in large part of cell-wall substance.

One form of crystal may occur in a given cell—the usual condition where crystals are clustered—or two or more types may be found in the same protoplast. Many inorganic crystals appear to involve organic matter in the course of their formation. The larger crystals of xylem often show this condition (Fig. 1.15 OHKL), and druses frequently contain a prominent organic center (Fig. 1.15 F).

The large crystals of somewhat various shapes—prismatic, rhomboid, etc.—occur chiefly in fibres of xylem and of phloem and in parenchyma cells associated with fibres, Raphides occur in thin-walled, mucilage-containing parenchyma-cells of soft tissues, such as storage parenchyma of underground parts,



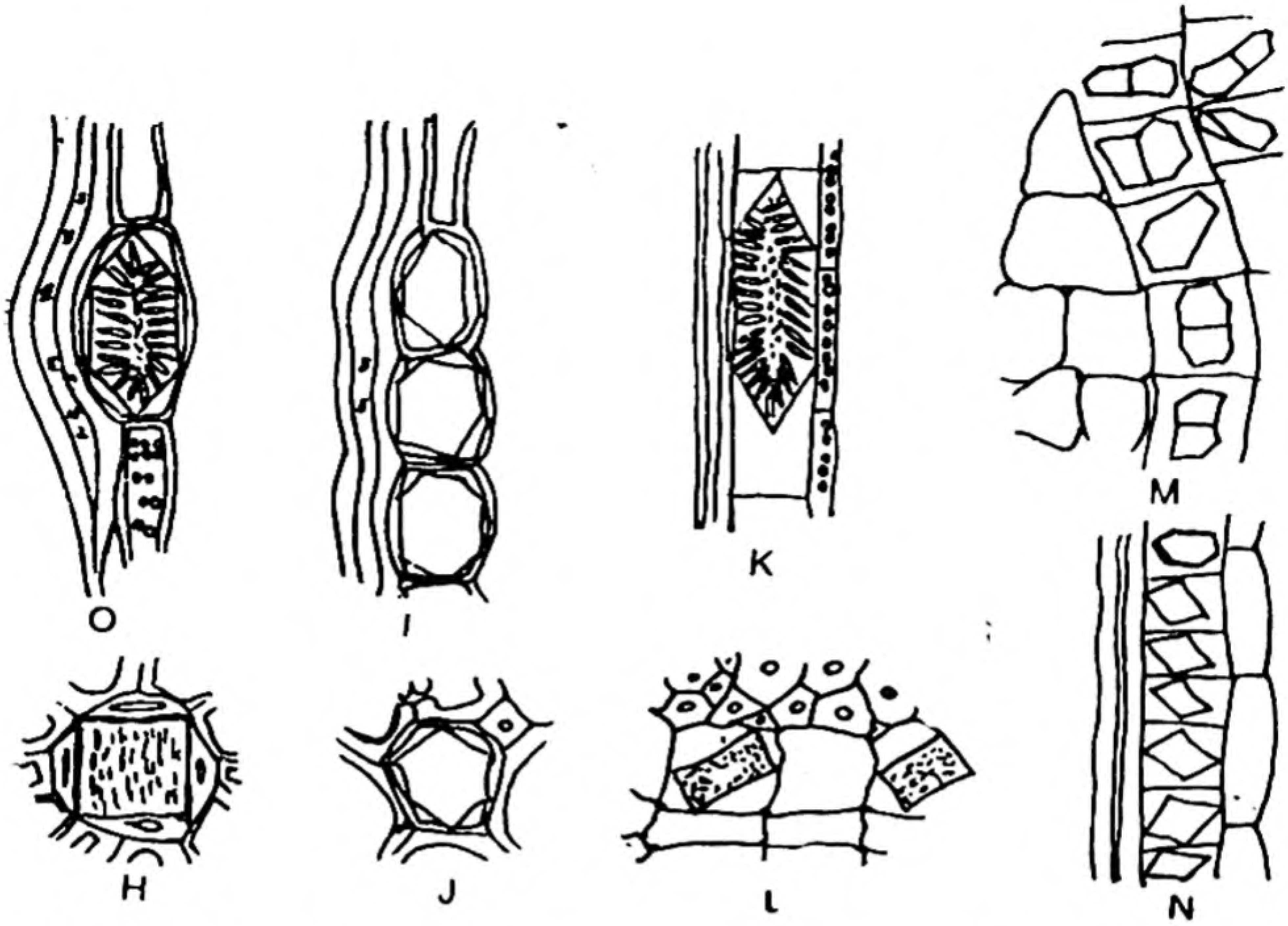


FIG. 1.15: Crystals. A druse in cortical cell of stem of Viburnum Lentago; B, druse and rhombohedral crystals in stone cells of nutshell of Carya glabra; C. solitary and grouped crystals in pith cells of Populus grandidentata; D. druse, in cortical cells of Carit Papaya; E, "bundles" of raphides in pulp cells of fruit of Smilacina racemosa; F, druse with organic center, in phloem parenchyma cell of Juglans nigra; O.H. longitudinal; an transverse sections of crystal in wood parenchyma of Carya Pecan; I.J. longitudinal and transverse sections of crystals in wood parenchyma of Juglans nigra; K.L. longitudinal and transverse sections of crystals in phloem parenchyma of Tilia americana; M. various forms of crystals in phloem parenchyma of Malus pumila; N, rhombohedral crystals in phloem parenchyma of Salix nigra.

fruit pulp, and the tissues of aquatic plants generally. They are present in monocotyledons chiefly. Druses are characteeistic of parenchyma cells of cortex and pith, especially of stems and petioles; they also are abundant in phloem.

Crystalslic in the protoplast– usually in the vacuoles—or in the lumen when the protoplast has disappeared, as in fibres.

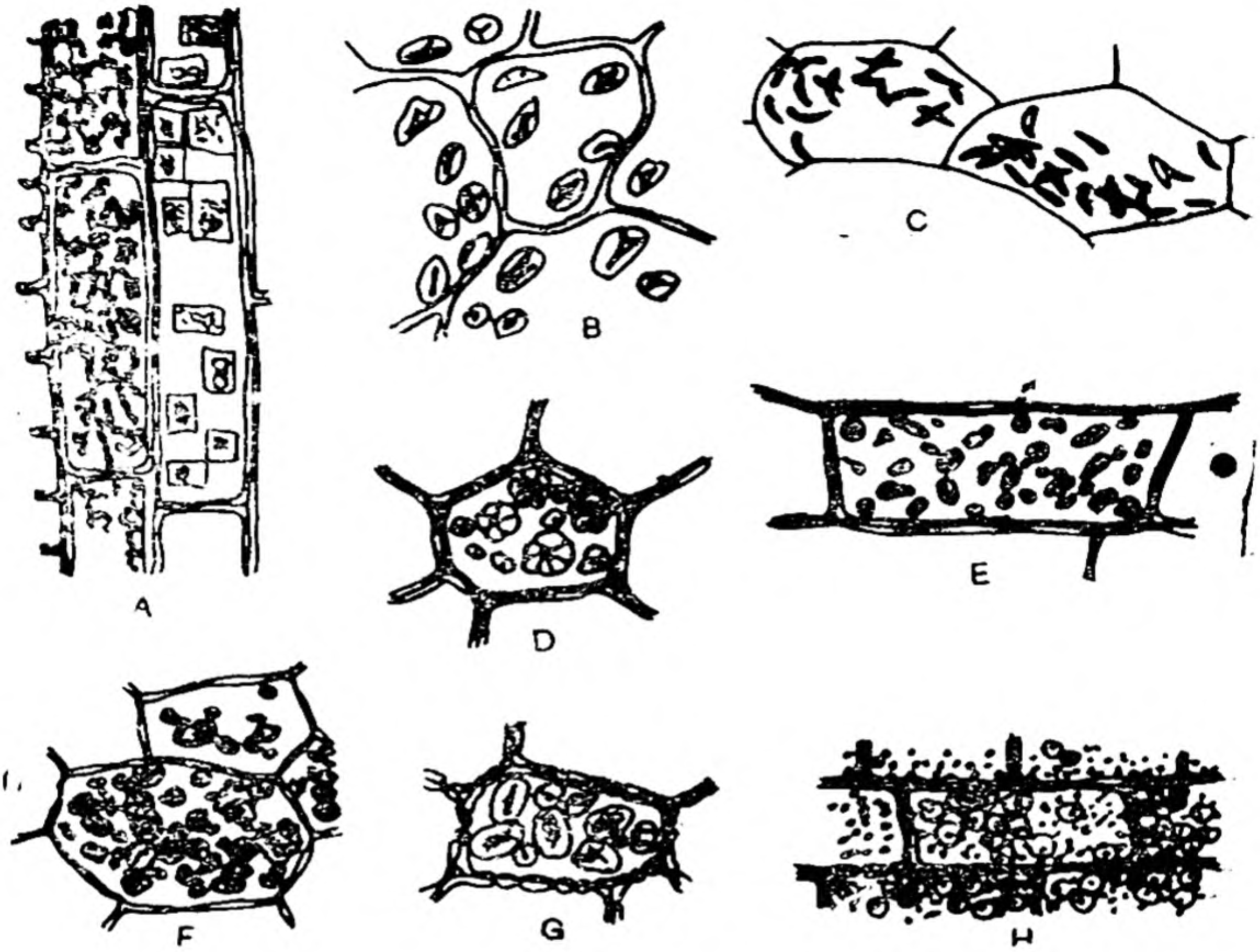


FIG. 1.16: Starch grains and tannin. Tannin: A, in phloem parenchyma of Pinus (also crystals); F, in pith cells of Fragaria; H, in ray cells of wood of Malus pumila (also starch grains). Starch grains: B, in pith cells of Alsophila; C, in outer pericarp of Mysa; D, in cotyledon of Pisum; E, in ray cell of phloem of Ailanthus; G, in cotyledon of Phaseolus.

Occasionally, they are partly or wholly embedded in the cell wall; more frequently they are suspended in the lumen by projections of the walls. These projections are rod-like; or they are sac-like, covering the crystal and holding it in a central position. Large crystals may fill the lumen and then determine the interior contour of the wall (Fig. 1.15 B); in elongate cells the lumen may be filled at a given level only (Fig. 1.15 K).

Cells may be given over entirely to crystal storage, and the protoplasts become much reduced or disappear, but typical active cells may also contain abundant crystals. The early stages of crystals are found in very young cells; apical meristems often contain young druses.

In large part, inorganic crystals are probably waste products, the result of metabolic processes. Their development in tissues which soon cease to be functional. such as pith. cortex, and secondary phloem, is suggestive of this.

Starch: Food materials are present either as transitory material or in the more or less permanent form of storage particles. Newly formed food may be in solution or exist as solid particles. Starch grains are the most common kind of solid food material found. They are of numerous types and vary in size and in form over a considerable range (Fig. 1.16). In shape they are mostly rounded or oval. Crowding results in the formation of angular shapes, and symmetrical, polyhedral grains are characteristic of some plants. Other plants possess compound grains which closely resemble simple grains, but show their compound nature when broken up into their constituent parts which are minute simple grains. All grains in a cell may be simple or compound, or both types may occur in the same cell (Fig. 1.16 D). Structurally the grain is made up of radially arranged tapering needles of amylose. The needles lie with their tips at the centre, their bases fused to form a border. Both the central area, the *hilum*, and the border are highly refractive. Concentric layering about the hilum is commonly seen, although this may be obseure without special treatment. The layering may become strongly excentric in the larger grains of some plants, for example, the potato. The layered appearance is probably the result of variation in the amount of water in the amylose; and this variation is due to alternations in growing conditions—in light intensity, temperature and humidity. Grains formed under constant growth conditions are not lameliated. The hilam is rounded or angular, sometimes lobed, forked, or stellate, and is highly refractive to light. By the presence of hila and by staining properties, starch grains may be distinguished from plastids and other protoplasmic bodies, and from other solid particles.

Starch grains are first formed within chloroplasts and are often found in that position; leucoplasts in storage cells build up starch grains within themselves from translocated food brought from green cells. Starch grains are thus formed both primarily and secondarily within plastids. It is not known with certainly whether, when a grain is mature, it is freed from the plastid or is still surrounded by a very delicate layer of plastid substance.

NITROGENOUS PRODUCTS

These are reserve food materials containing nitrogen. There are two types of nitrogenous food materials *viz*., proteins and amino compounds. ***Proteins*** are very complex macromolecules having carbon, hydrogen, oxygen and nitrogen. Sometimes, in addition to these, sulphur and phosphorous are also present.

Proteins are soluble in strong acids and alkalis. Some of them are water soluble also. Chemically proteins are composed of monomers called ***amino acids*** which are considered the building blocks of proteins.

Proteins are stored in seeds or grains in the form of *alearone crystals* (Fig. 1.17). These are present in the endosperm of castor, pericarp of maize grain etc. Each aleurone grain (crystal) is a solid, oval or rounded body enclosing a small crystal called *crystalloid* and a small globular body called *globoid*. The crystalloid is proteinaceous and occupies the major portion of the grain, while the globoid consists of phosphates of calcium and magnesium and occupies the rest of the grain.

Amino Compounds occur in the form of amino-acids and amines in the cell sap. These are more frequently present in the growing parts rather than in storage tissues.

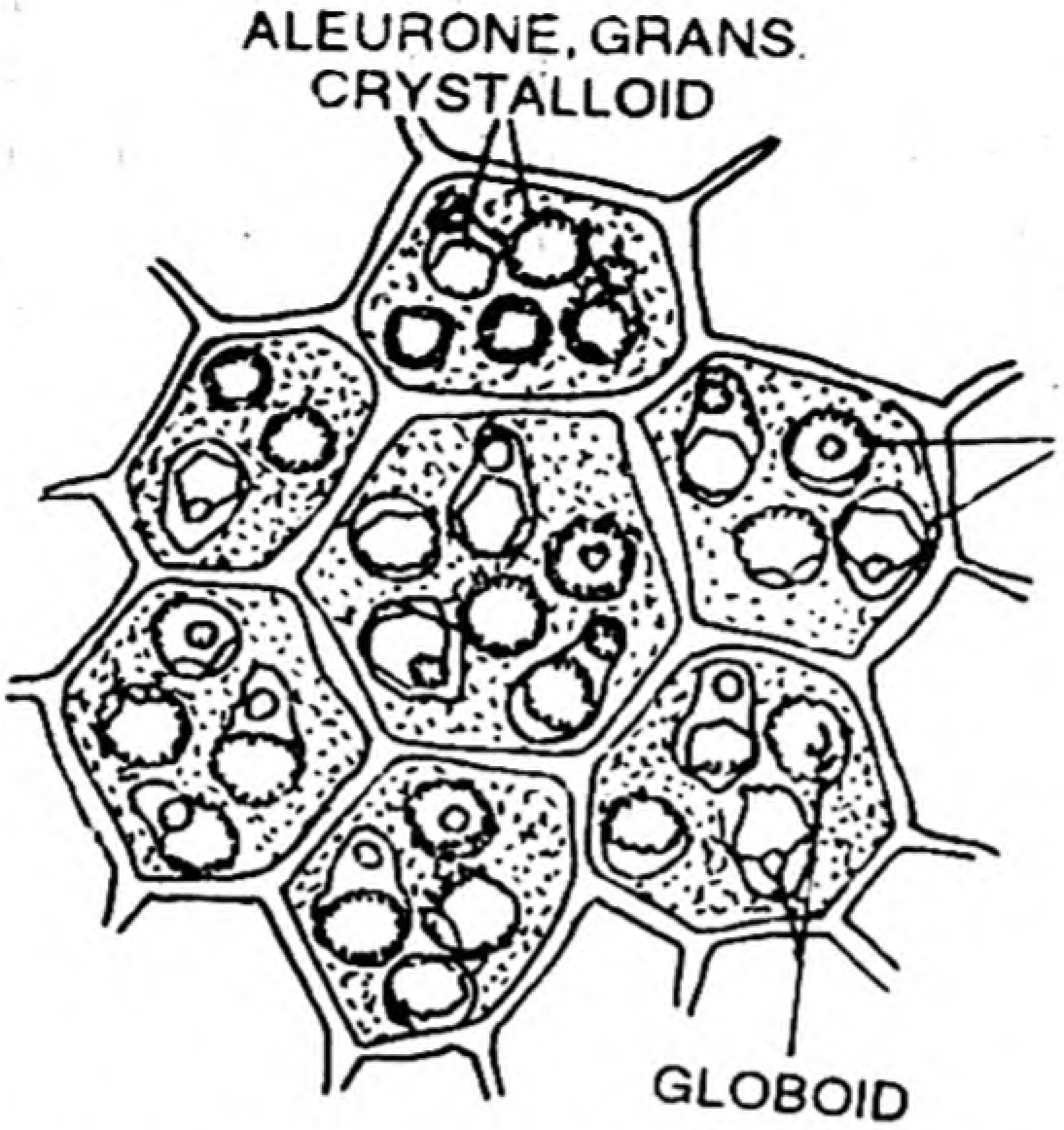


FIG. 1.17: Plant cell Aleurone grains in the endosperm of Ricinus communis (Castor).

Fats and Fatty Oils

Chemically fats are esters of fatty acids and glycerol. They occur in the form of oil globules in cytoplasm. They are present mainly in seeds. Chemically fats and fatty oils are composed of carbon, hydrogen and oxygen. They are insoluble in water but soluble in organic solvents. Fats store comparatively more energy than carbohydrates. Fats are solids at room temperature whereas oils are liquids at room temperature.

Secretory Products

In addition to the food materials the cytoplasm also secretes a number of chemical compounds which do not help either in nutrition or in growth.

Secretory products are of three types *viz.*, *Enzymes*, *Pigments* and *Nectar*. Enzymes are complex proteins and participate in various types of biological reactions. They are generally called organic catalysts, because they are not used up in a reaction, but enhance its rate, *Pigments* are colouring substances and are present in plastids or in the cell sap. These are usually present in leaves, and in the petals of flowers. Anthocyanins are the principal pigments which impart different colours to the petals. Chlorophyll, xanthophyll and carotenoids are pigments present in photosynthetic organs. *Nectar* are sweet substances secreted by special glands called nectaries which are present in flowers.

Waste Products

During the metabolic activity cytoplasm secretes various of types of waste products such as *tannins*, *mineral crystals*, *latex*, and *gums*. These are present either in cytoplasm or in the cell sap.

A. Tannins

They are non-nitrogenous substances found in the cell sap. Chemically they are derivatives of phenols and related glucosides. They are found in cell walls and also in the heart wood and the bark. Tannins are also found in leaves and unripe fruits. When the fruits ripen they are converted into glucose and other substances. Generally monocotyledons have poorly developed tannins. Tannins may be present in individual cells or in *tannin sacs*.

B. Alkaloids

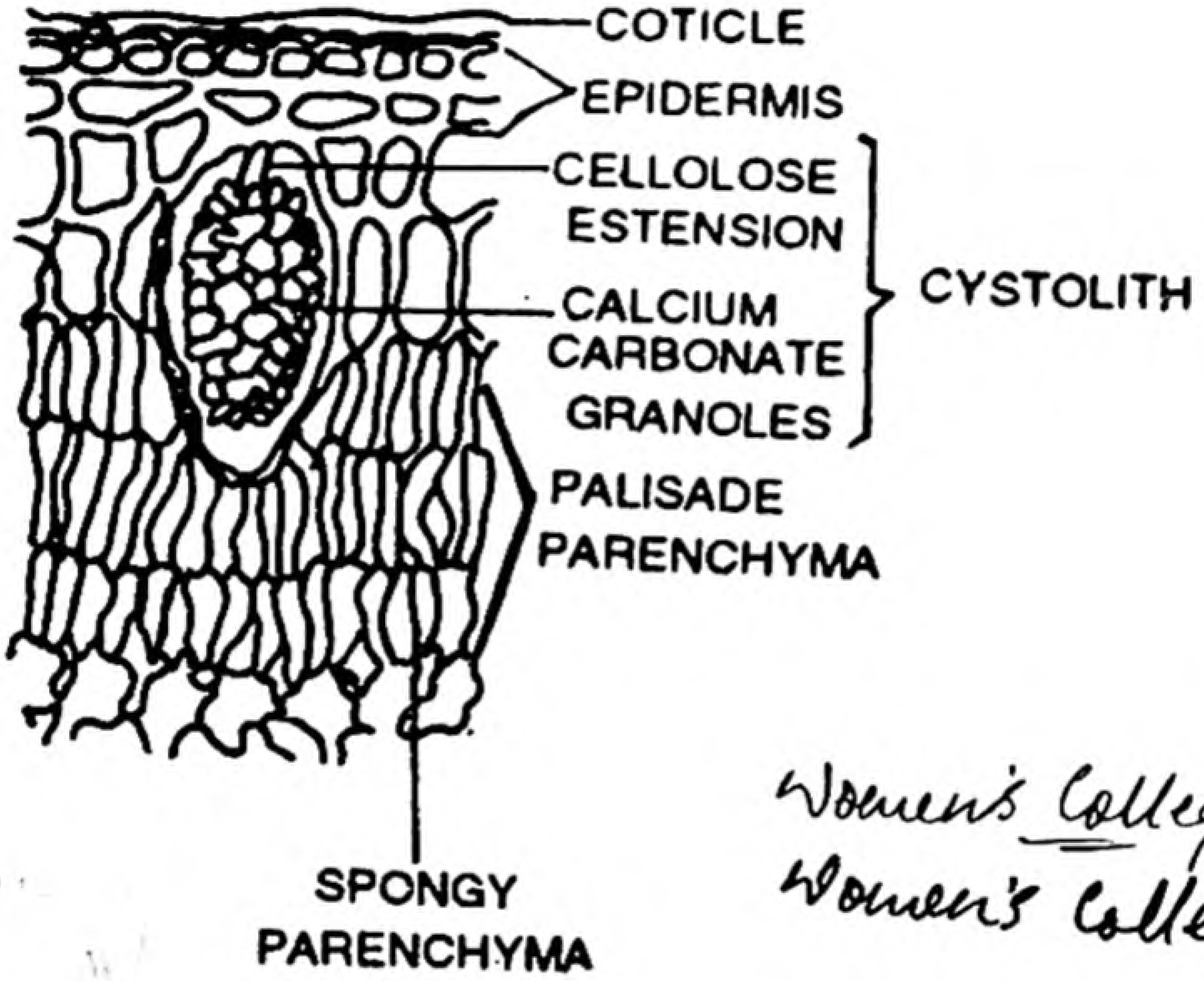
These are non-nitrogenous products found in various parts of the plant. Some of the common alkaloids are *Nicotine* (Tobacco), *Coffeine* (coffee), *Quinine* (Cinchona), *Ephedrine* (Ephedra), *Theaine* (Tea) etc.

C. Mineral Crystals

Various types of mineral crystals are found in plant cells. They may be present either in cell walls or in a cavity in the cytoplasm. Majority of the crystals contain calcium carbonate or calcium oxalate. The following are some of the types of mineral crystals.

1. Cystoliths

These are crystals of calcium carbonate, generally found in the leaves of various flowering plants. The members of the family *Moraceae*, *Urticaceae* etc., have cystoliths. Each cystolith has a peg like projection of the cell wall (Fig. 1.18) into the cell cavity. Cystoliths consist of many small crystals grouped together like a bunch of grapes.


COTICLE
EPIDERMIS
CELLOLOSE ESTENSION
CALCIUM CARBONATE GRANOLES
CYSTOLITH
PALISADE PARENCHYMA
SPONGY PARENCHYMA

Women's College.
Women's College.

FIG. 1.18: Plant cell Calcium carbonate cystolith in the leaf of Ficus elastica.

2. Calcium Oxalate Crystals

There are found in the vacuoles and are of the following types.

(a) Raphides

These are long needle like crystals arranged parallely in the form of stacks or bundles (Fig. 1.19). These are found in sacs and when the sacs are reptured come out with a jerk. Raphides are commonly seen in the leaves of *Colocasia*, *Pistia* etc.

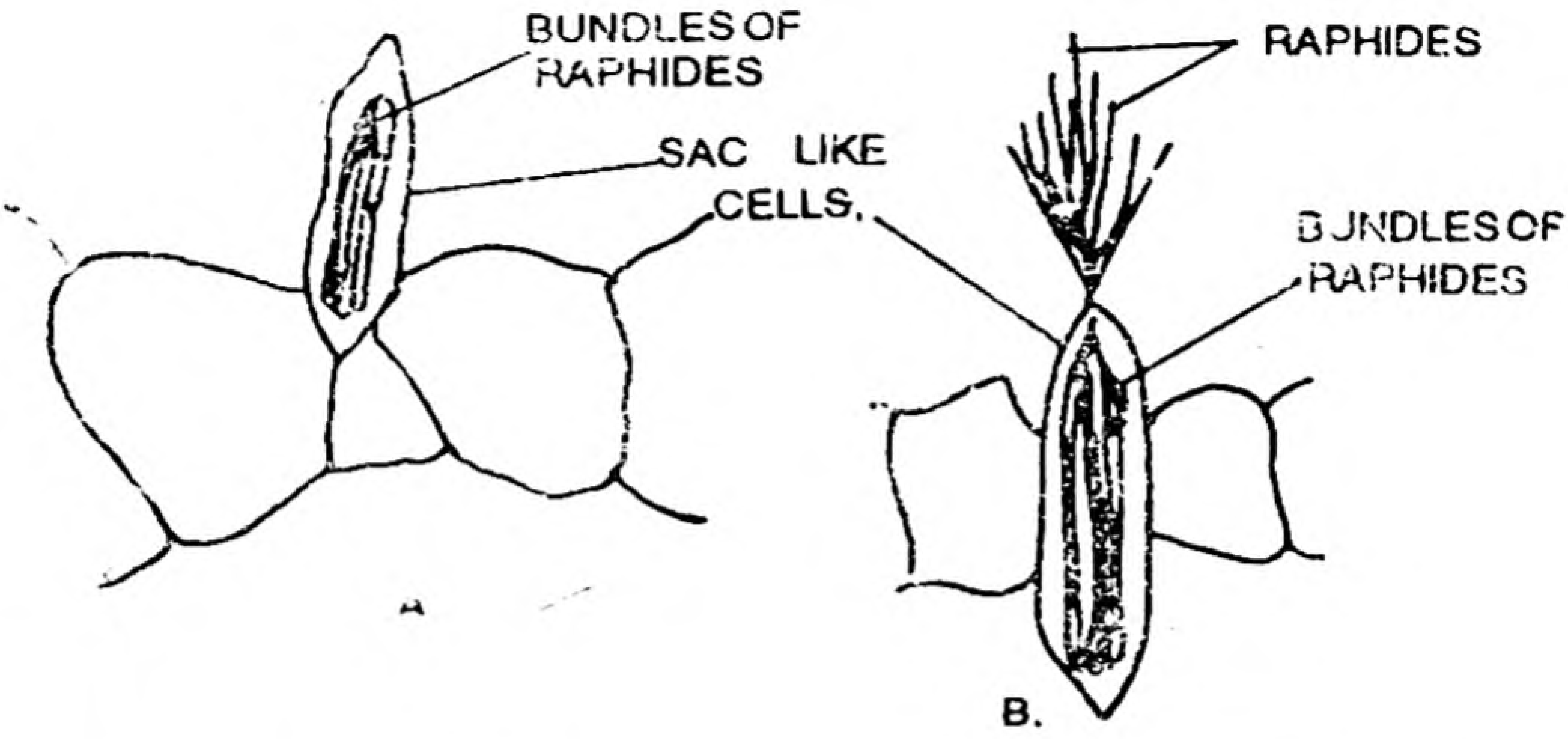

BUNDLES OF RAPHIDES
SAC LIKE CELLS.
A
RAPHIDES
BUNDLES OF RAPHIDES
B.

FIG. 1.19: Plant cell Raphides-A raphides in colocasla, B Projection of a raphide.

(b) Druses, Rosette or Cluster Crystals

These are compound crystals arranged in the form of a rosette (Fig. 1.20).

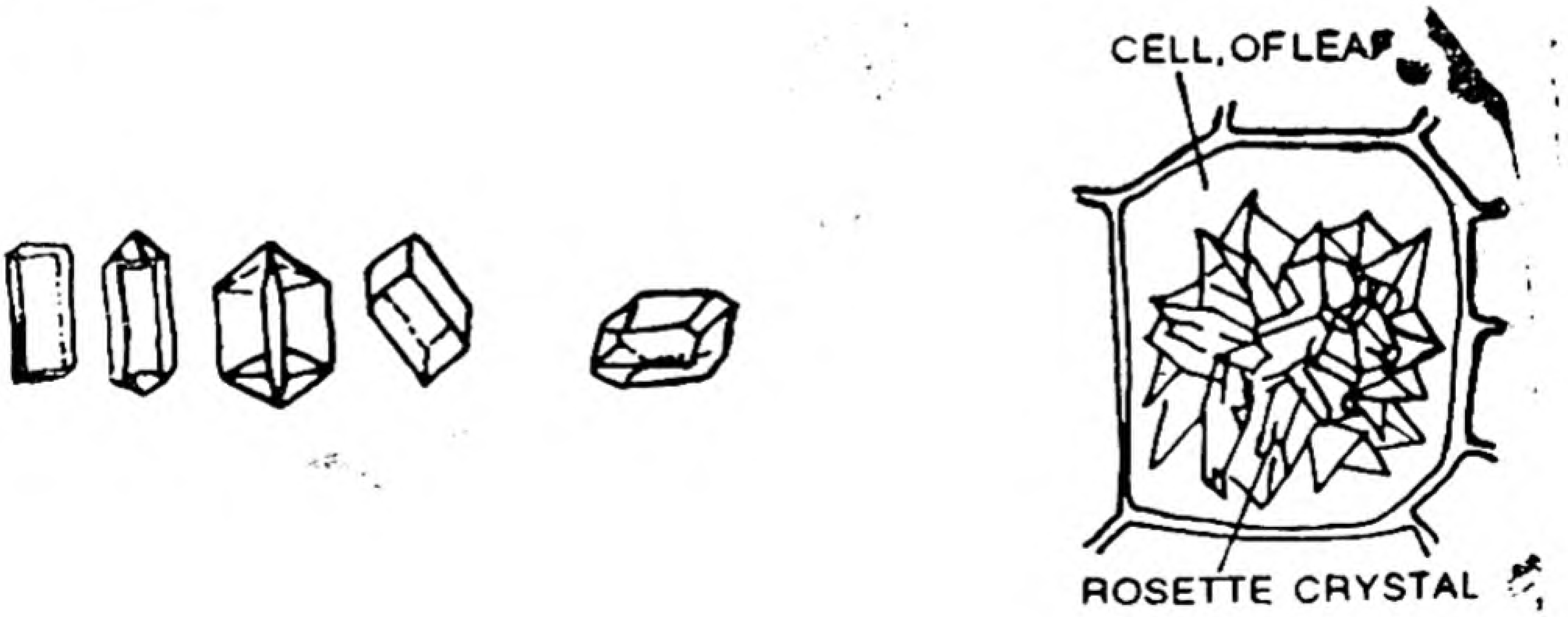

CELL, OF LEAF
ROSETTE CRYSTAL

FIG. 1.20: Plant cell Calcium oxalate crystals Prismatic crystals (left) and Rosette crystal (right)

These are found in the leaves of *Eucalyptus*, *Ixora* etc. Crystals found in globose masses are called druses. Shining crystals are called prismatic crystals (Fig. 1.20).

(c) Idioblasts

These are stellate crystals and are usually found in water plants (*Limnanthemunt*, *Nymphea* etc.) offering mechanical Support (Fig. 1.21).

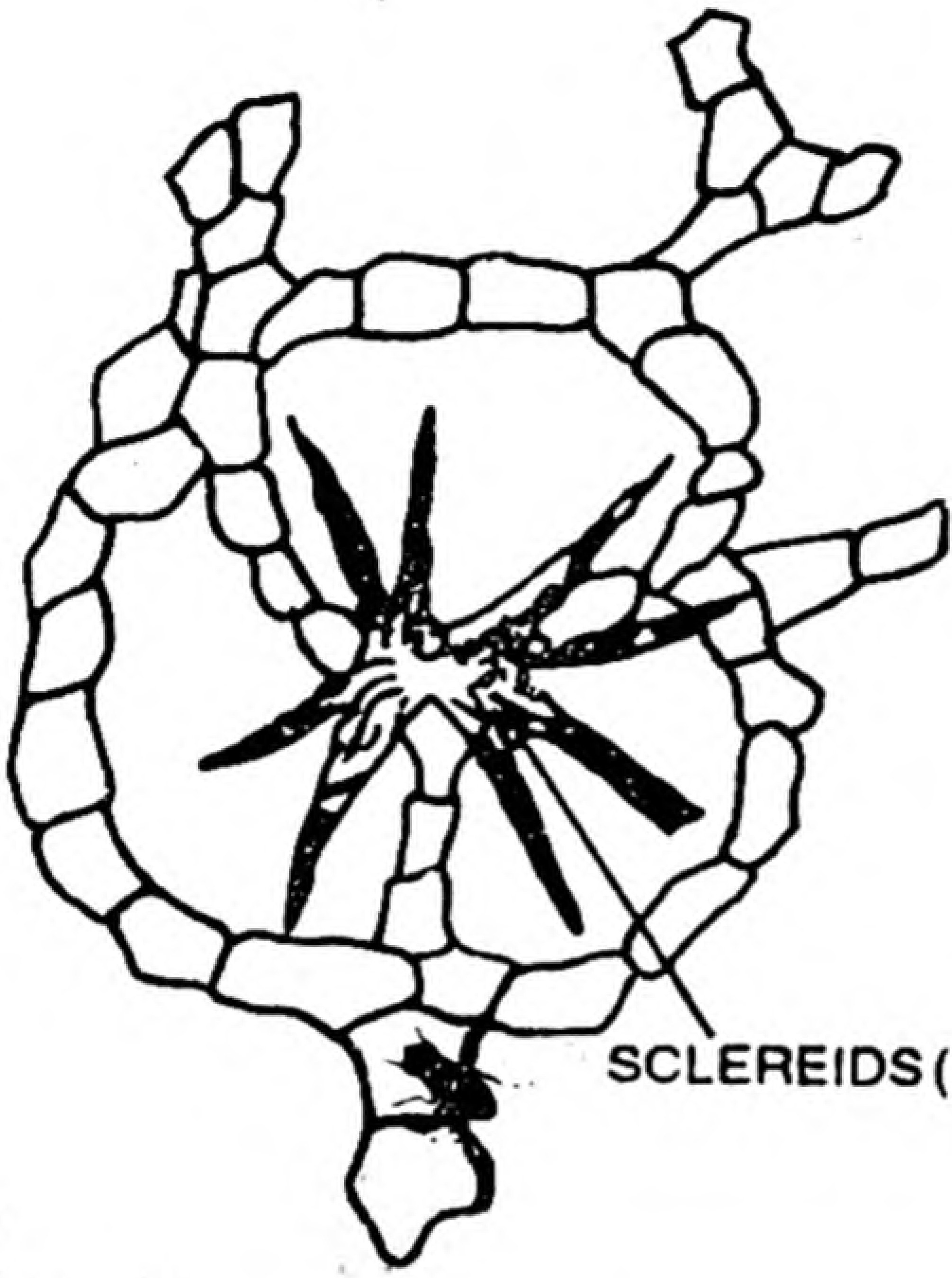


FIG. 1.21: Plant cell Idioblast in a leaf cell.

(d) Sand Crystals

These are found in masses packed into a cell. Such crystals are seen in the roots and leaves of *Atropa belladona* (Fig. 1.22).

(e) Latex

In some plants a milky or watery juice comes out of latex vessels of cells. Latex contain starch grams proteins, oils, tannins, gums, resins, alkaloids, salts, enzymes and many poisonous substances. Laticilerous cells are seen in *Carica, Euphorbia, Ficus Ilevea, Calatropic* etc. In some ceses the dried and processed latex is the source of natural rubber.

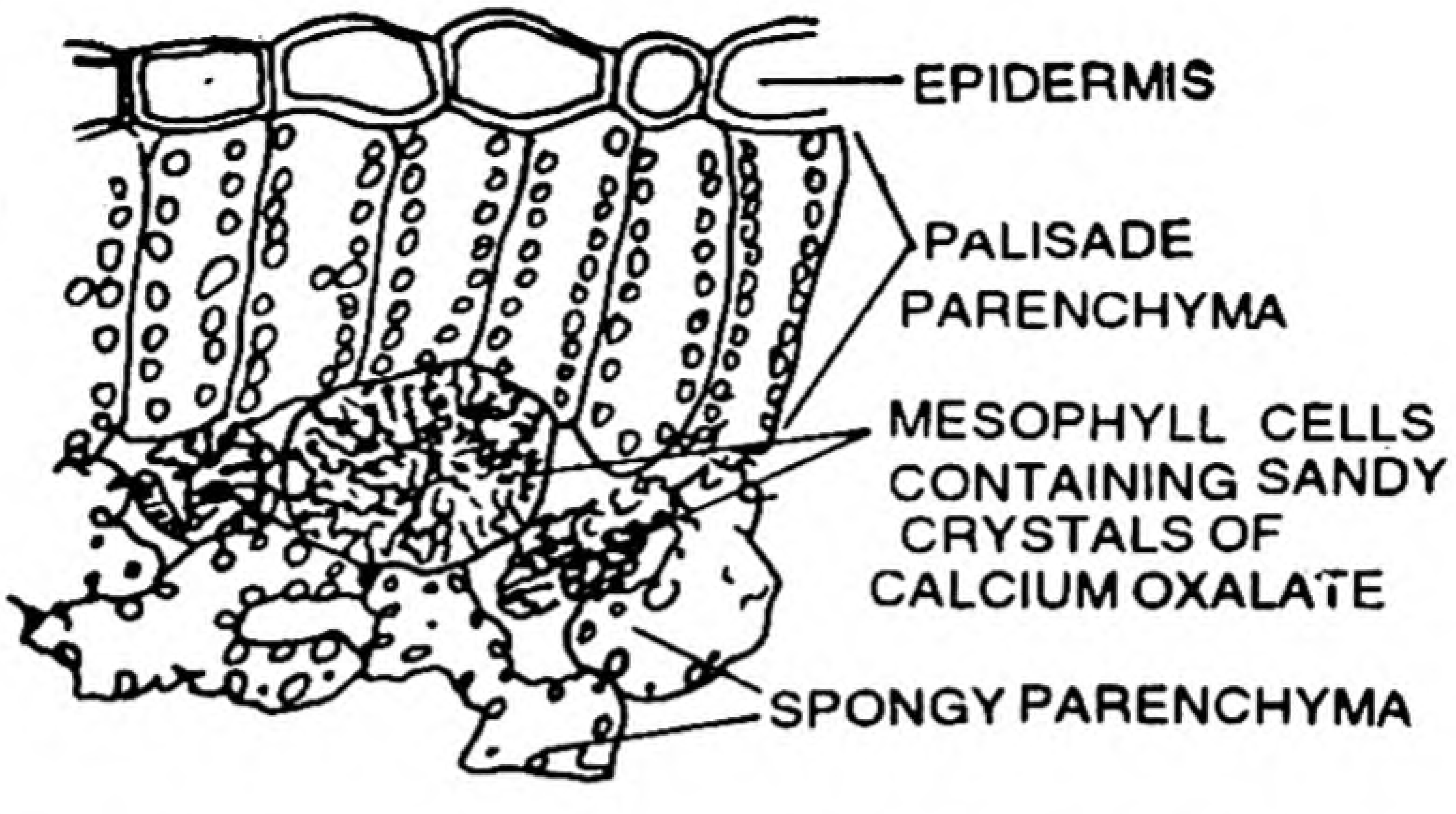

EPIDERMIS
PALISADE PARENCHYMA
MESOPHYLL CELLS CONTAINING SANDY CRYSTALS OF CALCIUM OXALATE
SPONGY PARENCHYMA

FIG. 1.22: Plant cell Sand crystals of Atropa belladona.

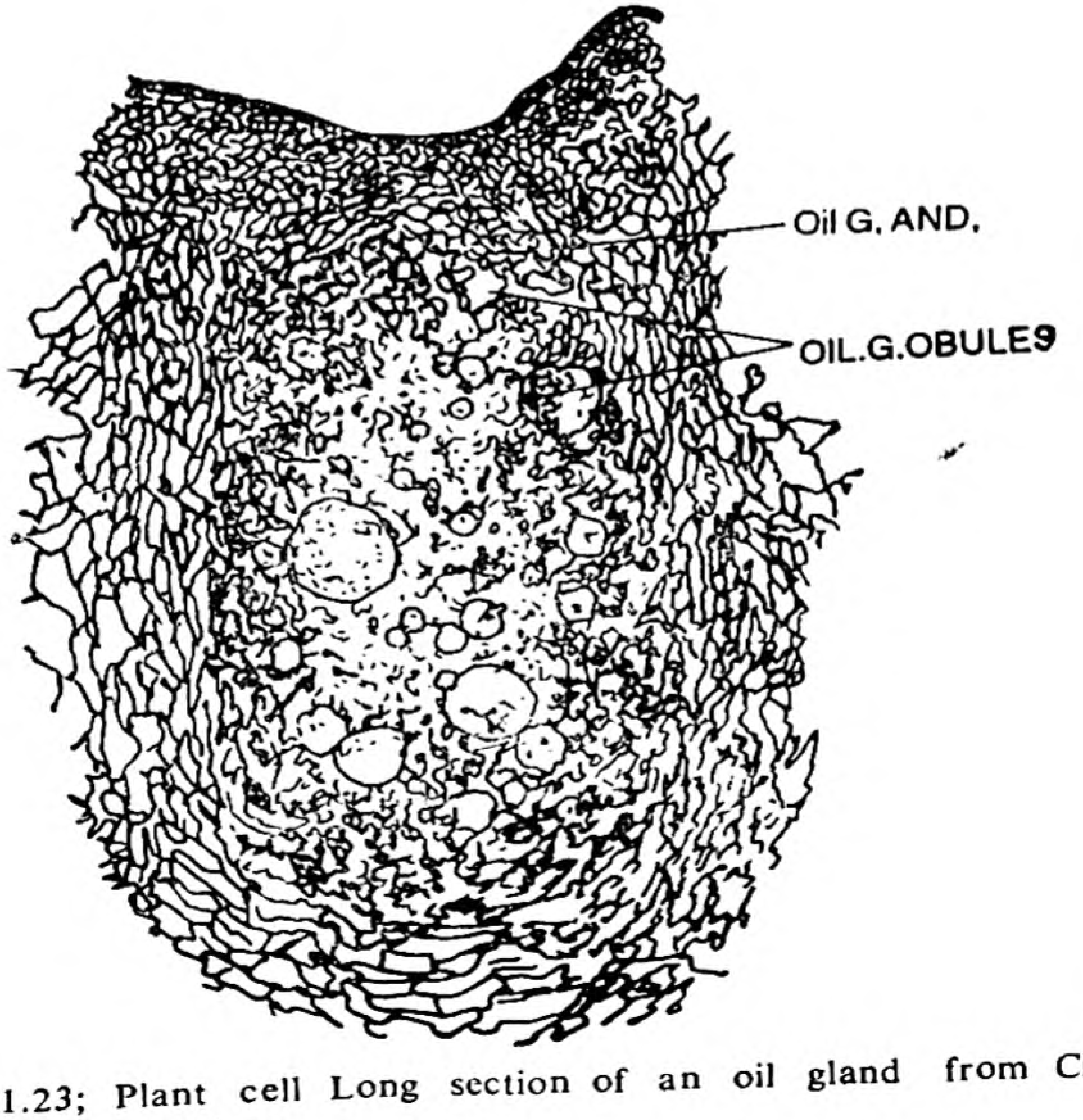

Oil G. AND.
OIL.G.OBULES

FIG. 1.23; Plant cell Long section of an oil gland from Cirtus aurantiaca.

(f) Essential Oils

These are volatile oils and are secreted by oil plants. Oil glands are found in almost all parts of the plants, specially in leaves (Fig. 1.23) and in the skin of fruits (*citrus* fruits). Essential oils are also found in the petals of Rose, Jasmine etc.

(g) Gums and Resins

These are produced in Gymnospeims (*Pinus*) and Angiosperms (*Acacia*, *Feronia* etc). Canada balsam a widely used resin in microscopic studies is produced from *Abies balsamia*.

2

Cell Wall

THE CELL WALL

In vascular plants only certain cells related to reproductive processes and early embryology are naked; all others have cell walls. This wall, at first very thin and delicate, is modified in various ways as the cell matures. The more important changes are increase in extent and in thickness; in chemical nature; and in modification of grosser, physical structure, such as the absorption of the end walls of vessels. The presence of a prominent wall, especially after the earliest stages of development, makes cellular structure in plants distinct—a feature in strong contrast with the condition in animals where the limits of the protoplast are less readily discernible.

In recent years the origin and structure of the cell wall have received critical attention and are now much better understood. A new interpretation is placed on the obscure earliest stages of wall formation, the structure of the wall is known in detail, and necessary changes have been made in the terminology applied to the wall.

Nature of the Wall

The cell wall is commonly looked upon as a secretion of the protoplast, laid down upon its surface. Strictly as such, the wall would be a nonliving layer. Although this is the view now generally accepted, the opinion is held by some that the wall in early stages, and the layers contiguous with the protoplast in later stages, may contain protoplasmic material and therefore form a part of the living unit. The middle lamella may also contain living substance in early stages and possibly as long as the protoplast remains active. If the wall is a living adjunct of the protoplast, certain facts of structure and behaviour of cells

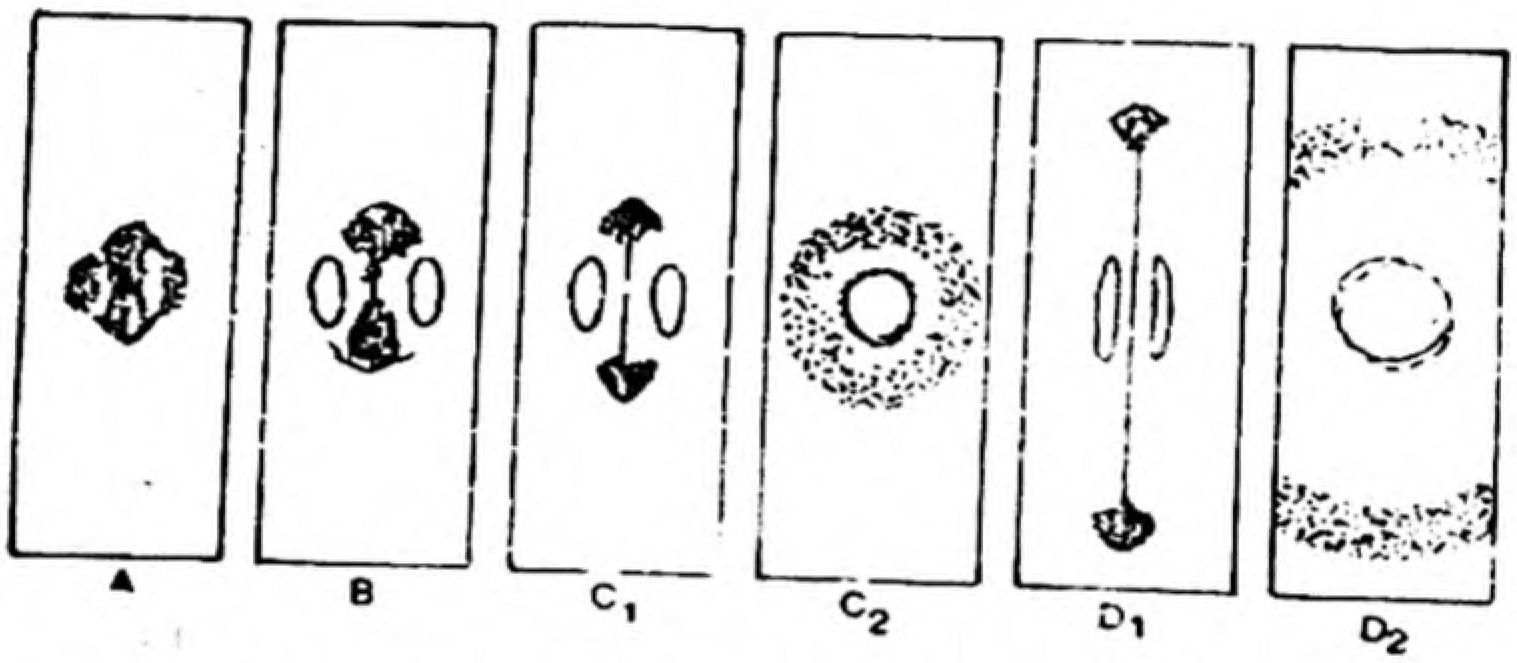


FIG. 2.1: Diagrams to show cell-wall formation during cell division of elongate cells, as seen in longitudinal sections. A, at late telophase stage: the phragmoplasts enlarging equatorially, with droplets of cell-plate substance forming along the median line; the spindle fibres disappearing in the polar and central regions. B, the beginning of the cell plate (lying at right angles to the page), formed by fusion of the droplets in the centre of the phragmoplast; new fibres forming on the equatorial periphery of the phragmoplast, the older ones progressively disappearing. C1, the phragmoplast, now an annular band, continuously expanding peripherally, has extended the cell plate in all directions. C2, section at right angles to C1, the phragmoplast (lying in the plane of the page), has extended nearly to the mother-cell wall in the central part of the cell; the cell plate, a delicate membrane in the plane of page, not shown. D1, the cell plate and phragmoplast ring extended much farther than in C1, D2, section at right angles to D1, the phragmoplast ring has reached the lateral walls in the central part of the cell and disappeared there, leaving two arcs (kinoplasmasomes), which will continue to progress to the ends of the cell; the cell plate has also reached the lateral cell walls in the centre, dividing the cell in the central part, while both ends remain undivided.

are more readily understood whereas others are difficult of interpretation.

Origin of the Wall

Wall formation is first evident at the close of nuclear division. As the daughter nuclei reach the telophase, the spindle fibres become stronger along the equatorial line and weaker near the nuclei (Fig. 2.1). With the surrounding, somewhat denser cytoplasm (the kinoplasm) they form a rather poorly limited, bulging-barrel-shaped structure, the *phragmoplast*. Along the median line of the figure, droplet-like particles appear, increase in size, and merge to form a fluid *plate* that divides the phragmoplast (Fig. 2.2). The plate is first evident at the centre of the phragmoplast and then extends marginally in all directions toward the mother-cell wall. As the plate forms, the central spindle fibres disappear, and with the extension of the plate, the peripheral spindle fibres also progressively disappear —the outer ones persisting longest. When the plate is complete, reaching the wall on all sides, its substance becomes less fluid,

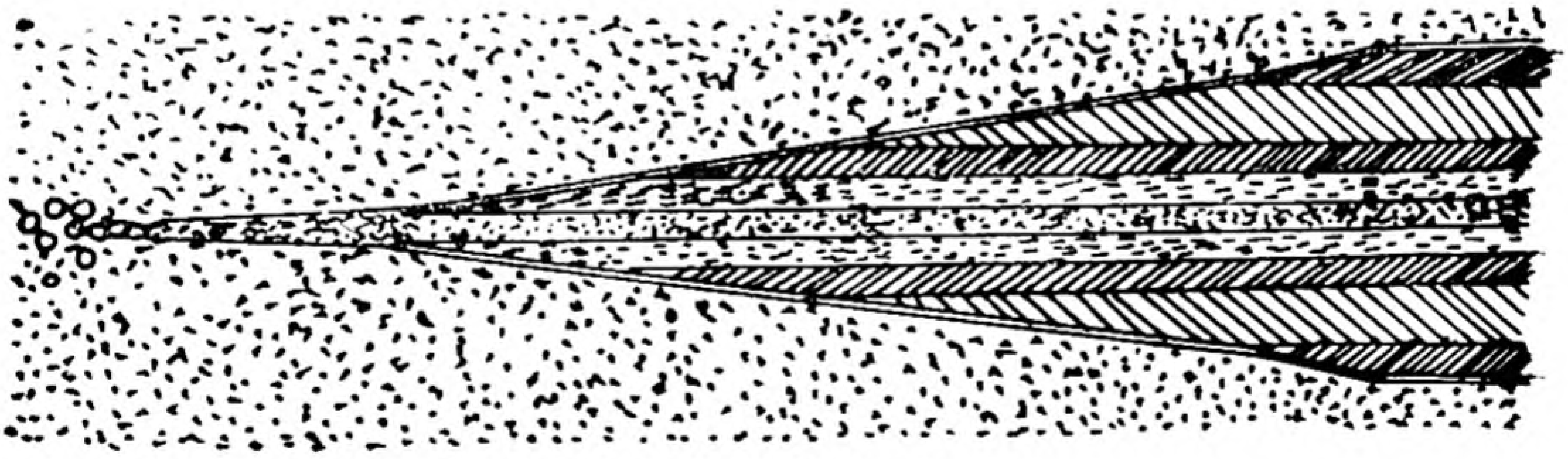


FIG. 2.2: Diagrammatic representation of successive stages (left to right) in formation of cell wall with secondary layers. (Walls of both daughter cells are shown.) a, origin of cell plate; b, beginning of transformation of cell plate into middle lamella (ml); c, beginning of deposition of primary wall (1, 1); d, e.f. beginning of deposition of outer, to middle, and inner layers (o, m, i) of secondary wall (2, 12); g, completion of wall thickening; pm, plasma membrane; cyt, cytoplasm. (Based on researches of W.A. Becker, I.W. Bailey, T. Kerr, and others; from Sharp.)

and delicate membranes appear on both its surfaces. These membranes are probably deposited by the daughter protoplasts. The constitute the first stages of the new cell walls of the daughter cells. The cell-plate material is gradually transformed into intercellular substance, which becomes an *intercellular layer*. The nature of the substance forming the cell plate is unknown; it is possibly cytoplasmic, and the spindle fibres may be "lines of flow" in the cytoplasm.

Where the mother cell is small and isodiametric and the nucleus large and central, the division figure is large, and no complex structural conditions are involved in the division of the protoplast. But cell division and the completion of the new wall are frequently much more complex. Division in cells with a large central vacuole involves predivision changes in form and position of nucleus and in structure of the vacuome (Fig. 2.3)—the cytoplasm increases in amount, extending in strands through the vacuole; the nucleus becomes more rounded and moves away from the wall along one of the cytoplasmic strands; the strands gradually become concentrated in a layer (the "phragmosome") in the plane in which the new wall is to be formed. Nuclear division and wall formation that take place much as in cells with abundant cytoplasm.

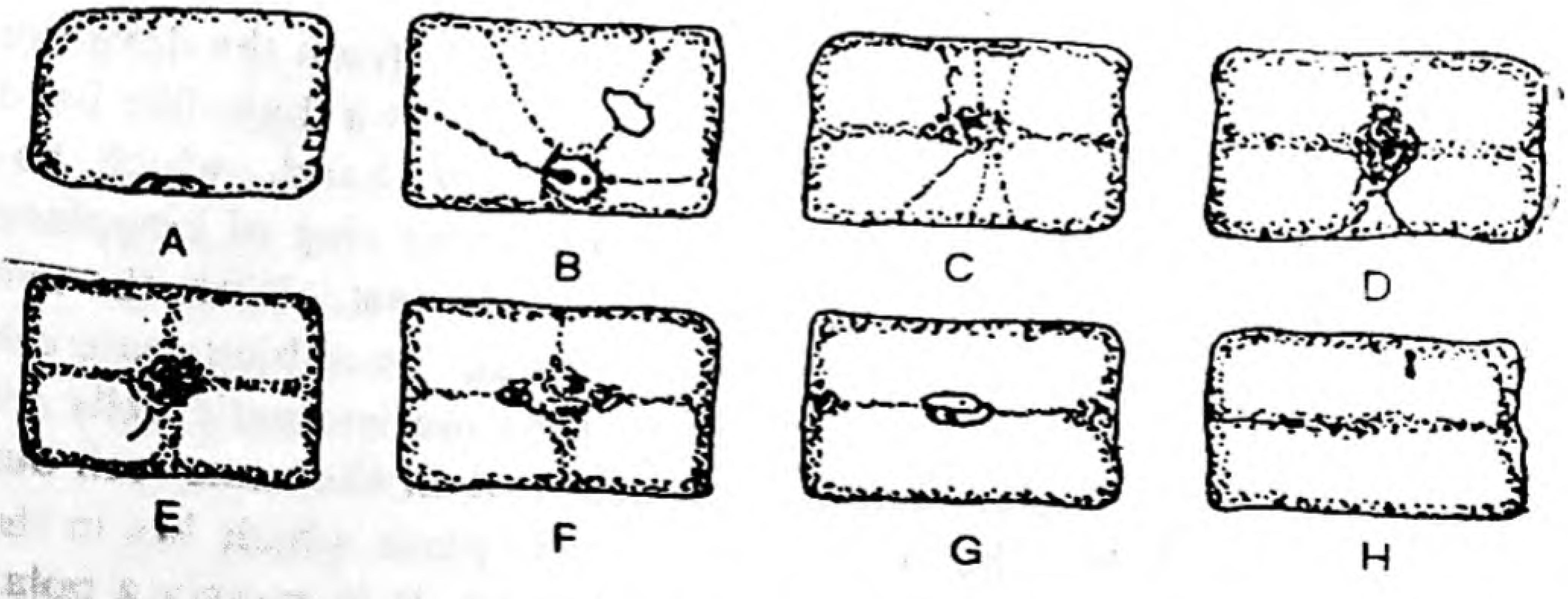


FIG. 2.3: Division in cell with large central vacuole. Cytoplasm increases in amount forming layer in position of new wall; nucleus becomes globose and migrates to central position; normal nuclear division with extending phragmoplast. (After Sinnott and Block, from Sharp.)

In cells in which one diameter is markedly greater than the others, especially in those that are very long, and when division is longitudinal, as in cambium cells, the phragmoplast persists long after nuclear division is complete and builds the cell plate far beyond the daughter nuclei and to the ends of the mother cell (Figs. 2.1, 2.3). Its polar parts and the central fibres disappear, as in isodiametric cells, with the formation of the first part of the cell plate (that between the daughter nuclei) and the plate increases in diameter, expanding as an equatorial belt in all directions. New fibres are added progressively at the periphery as the older ones disappear. The new fibres are short, forming only close to the plate, and recurve toward the nuclei (Fig. 2.1 C, D). The barrel-shaped phragmoplast thus becomes a continuously expanding, annular band of short fibers and dense cytoplasm. As the band expands, it is broken up into arcs (which have been called "kinoplasmasomes") as the parts that reach the nearer walls disappear. The persisting arcs continue to progress to the farthest parsts of the cell, adding to the cell plate on its margins until the cytoplasmic body is completed divided. The constant presence of the fibres as the plate is formed and their renewal on its progressing margins far from the nuclei suggest close relation of these structures to the building of the plate.

As the phragmoplast ring moves away from the daughter nuclei, it appears, when seen in face view, as a halo-like band surrounding the two nuclei. This circular band, which has been called the "phragmoshere," is merely the ring of kinoplasm which at this stage constitutes the phragmoplast. With the two nuclei, it has been mistakenly interpreted as a binucleate cell and made the basis for the statement that meristematic cells are commonly binucleate. The nuclei are not in the same cell but are separated by the very young cell plate which lies in the plane of section and is not readily detected. It is merely a polar view of a late stage in cell division. Longitudinal cross-sections show the phragmoplast as two rounded or wedge-shaped masses at the ends of the weakly developed cell plate (Fig. 2.1C, D).

In such cells as cambium initials, procambium cells, young sieve-tube elements, young fibres, and other elongate cells, cell-plate extension is of extraordinary degree and the wall often of unusual form. Curved and even cup-shaped walls may be built by the changing plane of the ring as the plate is formed. The breaking up of the phragmoplast ring into arcs and the slow completion of the division of the protoplast are common but neglected features of cell division. This represents is a special type of cell division but an adaptation to cell form.

Gross Structure of the Wall

In mature, thick-walled cells, a concentric layering is usually evident in the wall. The layers represent successive deposits of wall substance by the protoplast upon the cell plate. The layers differ from one another in physical and chemical nature, their capacity for adaptation to changes in cell size and shape, and in extent over the earlier formed wall. They fall into two major grups, constituting the *primary wall* and *secondary wall*. The primary wall usually consists of a single layer. The secondary wall is made up of one to many layers, more frequently of three.

The Primary Wall

The membrane formed on the surface of the cell plate is the first stage of the primary wall. As cell enlargement begins, this membrane becomes somewhat unevenly thickened but remains delicate and mesh-like in finer structure. During rapid increase in area, the wall may maintain its thickness and unevenness, or be alternately thickened and thinned. The uneven thickening soon sets of more or less distinct thinner areas, *primary pit-fields*, sometimes known as *primordial pits*. Primary pit-fields are conspicuous in cambium initials. At this stage abundant plasmodesmata, protoplasmic connections between the protoplasts separated by the new walls, are evident, clustered chiefly in the primary pit-fields. The presence of large numbers of plasmodesmata perhaps determines the location of the pit-fields. The mesh-like earlier wall is probably also freely penetrated by cytoplasmic projections, the protoplasts remaining in continuity from the division stage, but such connections have not been demonstrated. At this early stage the wall substance is possibly highly protoplasmic, as may also by the middle lamella.

The young wall remains plastic and adaptabled to changing volume and shape as the young protoplast continues growth. Inequalities in thickness persist, and alternate thinning and thickening may continue. With attainment of final size and form in the cell, the primary wall takes on mature structure and may thicken further. Small, well-defined thin areas, *pits* are formed, one or more in each of the primary pit-fields. The phasmodesmata are largely restricted to the pits, but seattered, even solitary, stands may occur outside these areas. The mature primary wall varies greatly in thickness and in chemical and physical structure in cells of different types and in cells of the same type in different tissues and different plants.

The Middle Lamella

As the first stages of the primary wall are developing, the substance of the cell plate becomes, by physical and chemical changes, a firm *intercellular layer*, a *true middle lamella*, which serves to bind the cells together. The term *middle lamella* has been in common use for a middle layer in the walls separating two protoplasts, or two cell lumina, that differs fromt he rest of the walls in staining and light-refracting qualities. Such a layer is, however, structurally and morphologically complex; according to this loose usage the "middle lemella" includes the true middle lamella (intercellular substance) plus the contiguous primary walls of the two cells and often also the thin, first-fomed layers of the secondary wall. The intercellular layer is the middle layer of this group of three or five layers and the term middle lamella should be restricted to this. In stained preparations the true middle lamella is commonly difficult to differentiate because of its close resemblance in chemical nature to the contiguous walls. For convenience, the term "middle lamella" will doubtless continue to be used for the compound layer, but it should be remembered that this is a loose and inaccurate use.

Along the line of contact of the new wall with the mother-cell wall the new and the old middle lamellas are separated by the primary wall of the mother cell (Fig. 2.4). Union of the layers of middle lamella is secured by the development, along the line of contact of the old and new walls, of a cavity—triangular in cross section—in the mother cell primary wall, which enlarges and extends to the middle lamella, and the extension into this cavity of the intercellular substance of the new wall. When the cavity continues to enlarge, and the intercellular substance does not fill it, an intercellular space lined by intercelluler substance is formed. Commonly the formation of intercellular spaces is more complex than this. More than two walls are concerned and the development of the interwall space is preceded, accompanied, or followed by a splitting of the middle lamella of the older wall nearby. The interwall space and the space formed by the splitting merge, and the cavity so formed enlarges by further splitting and by pulling away of rurrounding cells under growth stresses. Increase in size of spaces sometimes results from shrinkage of cells. When two new walls are formed opposite one another, the two triangular spaces merge—sometimes with a split which appears between them—and a diamond shaped space is formed. In meristems where cell divisions succeed one another rapidly and all the walls are thin, intercellular spaces seem to be formed by a simple splitting apar of cells through the middle lamella.

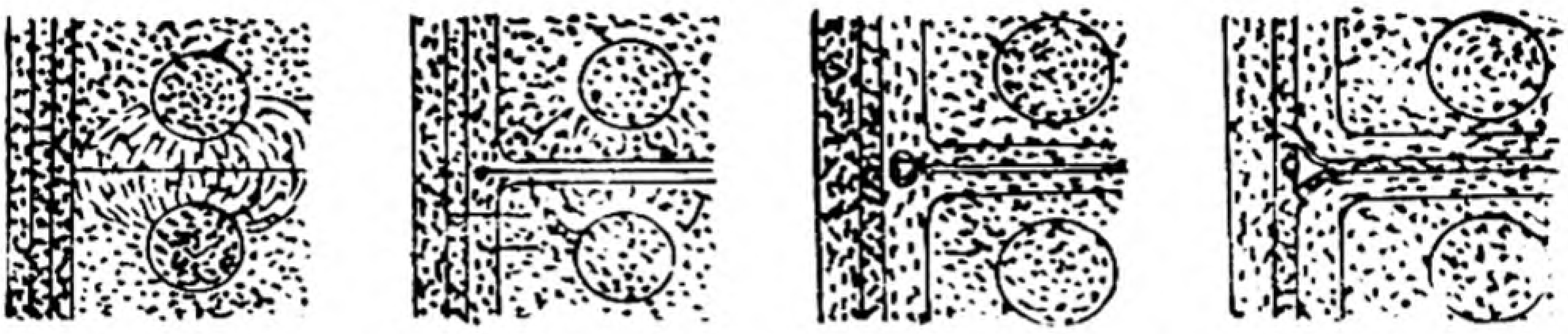


FIG. 2.4: Stages in the establishment of the connection between the middle lamella of a newly formed wall with that of the lateral wall of the divided cell, ml, middle lamella; l, primary wall; *cyt*, cytoplasm. (After Martens, from Sharp.)

The Secondary Wall

In many cell types further wall-thickening occurs after cell size and shape are fully attained. The wall then formed is the *secondary wall.* This differs from the primary wall especially in that it is incapable of increase in area and that such modifications as occur ars irreversible. The primary wall covers the protoplast except where the plasmodesmata occur; the secondary wall is formed over the primary wall except over the pit membranes. As this wall thickens, the pit cavities become deeper, and in some kinds of cells, partly enclosed and complex in structure (bordered pits). The secondary wall tends to be more massive than the primary, and in most thick-walled cells it constitutes the major part of the wall. In the tracheids and vessels of the protoxylem the secondary wall covers much less of the primary wall; it forms only as rings, spiral bands, and bars over the delicate primary wall.

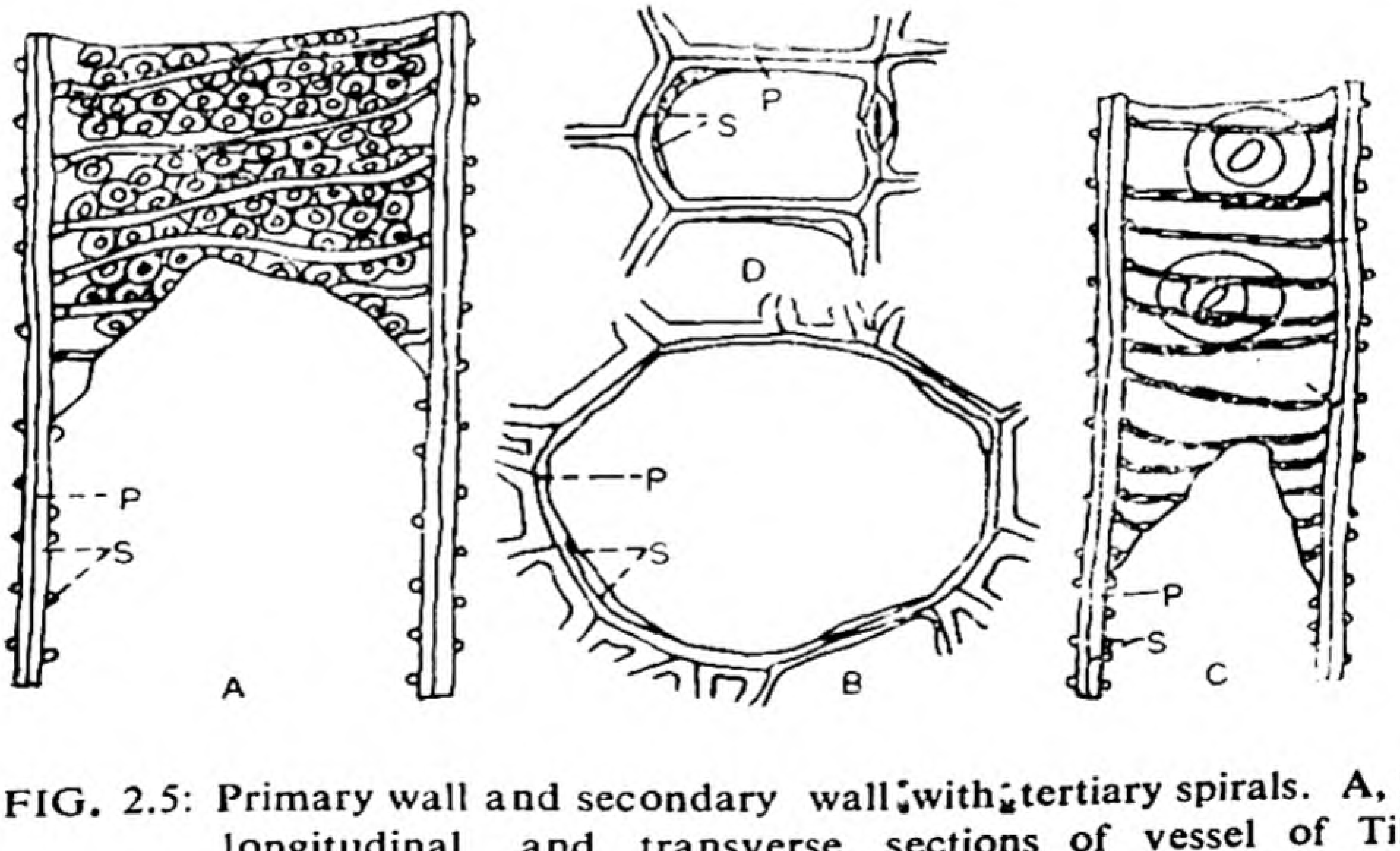


FIG. 2.5: Primary wall and secondary wall with tertiary spirals. A, B, longitudinal and transverse sections of vessel of Tilia americana; C, D, longitudinal and transverse sections of tracheid of Taxus brovifolia.

The last-deposited layer of the secondary wall of some wood elements—laid down over a wall with fully developed pits—has the form of delicate spiral bands (Fig. 2.5). Because these bands appear to represent thickenning after the secondary wall is complete they have been called a *tertiary wall*, but they represent only the innermost layer of the secondary wall and are now called *tertiary spirals* or *spiral thickenings*. The term "tertiary spirals" better distinguishes them from the spiral (secondary) bands of protoxylem which they somewhat resemble. Tertiary spirals lie upon a thick secondary wall; protoxylem spirals lie upon a delicate primary wall.

Minute Structure of the Wall and Middle Lamella

Both primary and secondary walls are composed fundamentally of a complex mesh-like matrix of cellulose. Other substances fill the interstices of this framework and may be replaced later by different ones. In the primary wall pectic substances accompany the cellulose in early stages; the secondary wall is at first composed. Lastly of cellulose or of cellulose and hemicelluloses. Substances that at later stages replace, or are added to these are lignin, cutin, suberin, various inorganic materials, tannin, oils, and many others.

In minute structure the cellulose matrix of both walls is made up of aggregates of delicate fibrils that grade down in size to the limits of microscopic visibility. The fibrils are believed to be made up of *micellae*, aggregates of molecules, probably in crystalline form. The fibrils form complex, three-dimensional anastomosing systems which cohere firmly and are continuous throughout a wall. Variations in size, number, and arrangement of the fibrils give different structural patterns. The pattern may be dominantly radial or dominantly concentric. It is probably usually a combination of these—radial arrangement intensified at intervals during formation by increase in number or in size of fibrils so that a concentric layering is apparent.

Within the meshes of this matrix, channels and pores form another system in which are deposited various substances. The substances filling the interstices form a 'secondary' structural system which in pattern necessarily follows that of the primary system. A wall may thus be built up, for example, chiefly of two substances such as cellulose and lignin, closely intermeshed. Either of these substances can be dissolved out leaving intact the system formed by the other. The orientation and size of fibrils and pores varies in similar cells, in different parts of a cell and in different layers of the same wall.

The fibrils are commonly oriented with their long axes parallel with one another and with the surface of the layer they constitute. When the aggregates of fibrils are sufficiently coarse they may be seen on the face of the cell wall as delicate *striations*. These lines are most frequently spiral but may run in any direction. The fibrillar arrangement varies in different layers of the secondary wall (Fig. 2.6), and that of the primary wall differs from that of the adjacent layer of the secondary wall and may aid in distinguishing these walls. In successive layers the fibrils commonly run in different directions, and the major lines of structure in a thick cell wall run at an angle with those of the abutting wall of the neighbour cell suggesting that such arrangement gives mechanical support. In fibre-trachedis and wood fibres, the long axis of elongate bordered-pit apertures is parallel with the fibril aggregates of the thick median layer of the secondary wall. The pit cavities lie between radial plates of fibrils. The form of pit apertures thus may give clues to the orientation of the fibrils in thick walls.

The mature primary wall of many cells remains thin. When young, uneven thickening may give it reticulate structure, and when mature, abundant small pits may make it appear sieve-like. When this wall is a place for food storage, as in endosperm and some sieve tubes, it becomes greatly thickened. Probably the thick wall of collenchyma cells, with its high percentage of water, is wholly primary. A distinction between a strongly thickened primary wall and a primary wall plus a secondary wall may be difficulty to make without special study of optical characteristics.

Layering is not usually apparent in primary walls except with special treatment. In wood cells, especially in heartwood, the primary walls of adjacent cells and the middle lamella between them may be chemically closely alike and difficult to distinguish. This triple layer, together sometimes with parts of the secondary walls, has for this reason passed, in loose use, as "the middle lamella."

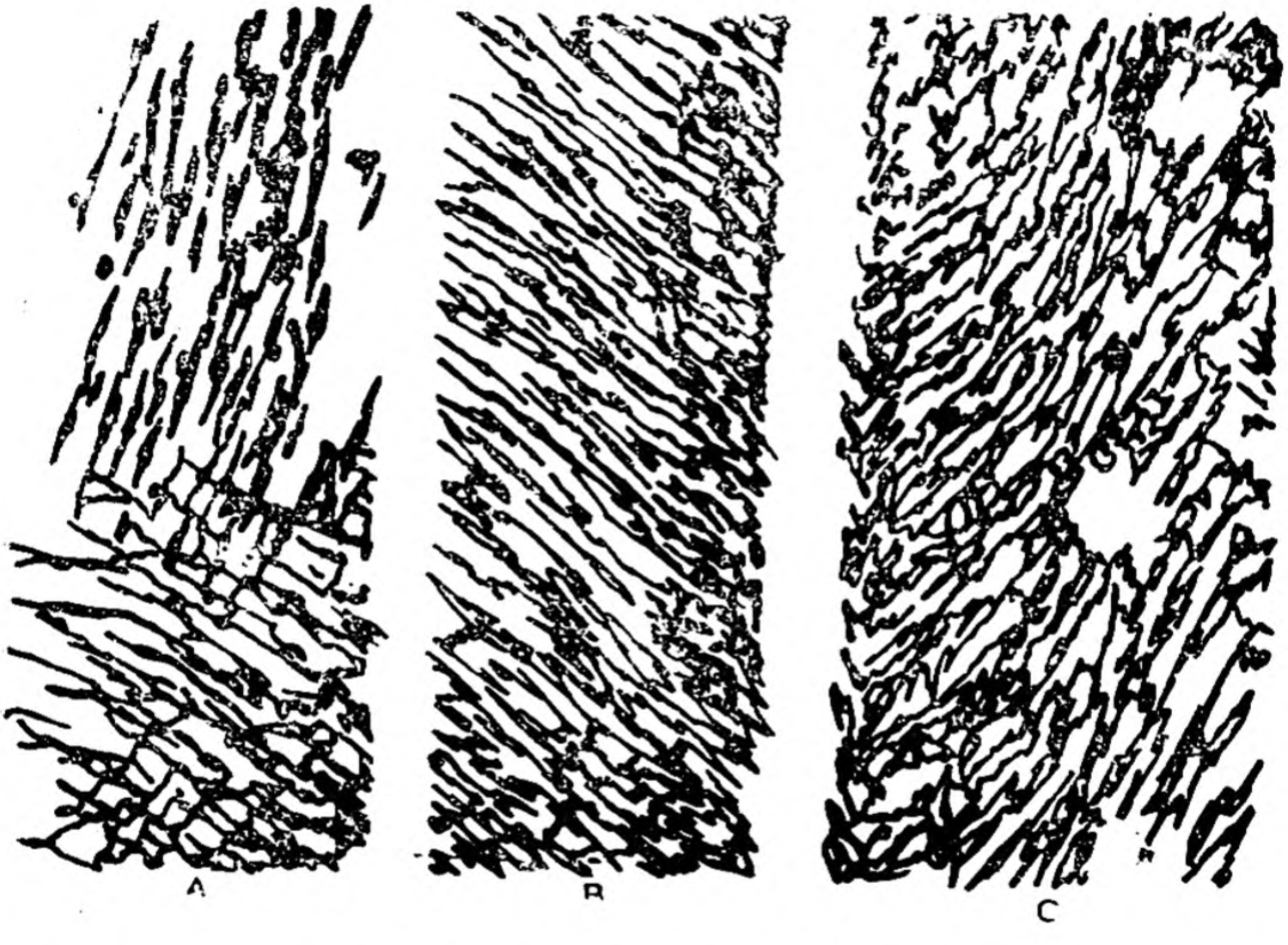


FIG. 2.6: Minute structure of the secondary wall. (Crystalline aggregates of iodine have been induced to form in the elongate interstices of the cellulosie matrix. The crystals are oriented parallel to the fibrils and so show variations in orientation of cellulose in the different wall layers.) Latewood tracheids of Larix occidentalls. A, lower part of figure, spiral arrangement in outer layers of two abutting cells, the fibrils crossing at right angles; upper part of figure the fibrils running nearly longitudinally. B, as in lower part of A, the crystals in the wall (of the adjacent cell) below showing faintly. C, derivations in the spiral arrangement due to the presence of bordered pits. (After Bailey and Vestal.)

The secondary wall, which constitutes the larger portion of the wall in most thick-walled cells, commonly shows marked lamellate structure, the layers ranging in number up to many. A division into three major layers is frequently seen, especially in wood cells: an outermost thin layer lying against the primary wall; a thick median layer; and an inner-most thin layer. Constituting the "wall" between two protoplasts or lumina among such cells, there are nine major layers—six secondary layers, two primary walls, and the middle lamella (Fig. 2.7). The heavy median secondary layer itself frequently shows many minor layers varying in distinctness. The function of the thick secondary wall is primarily mechanical.

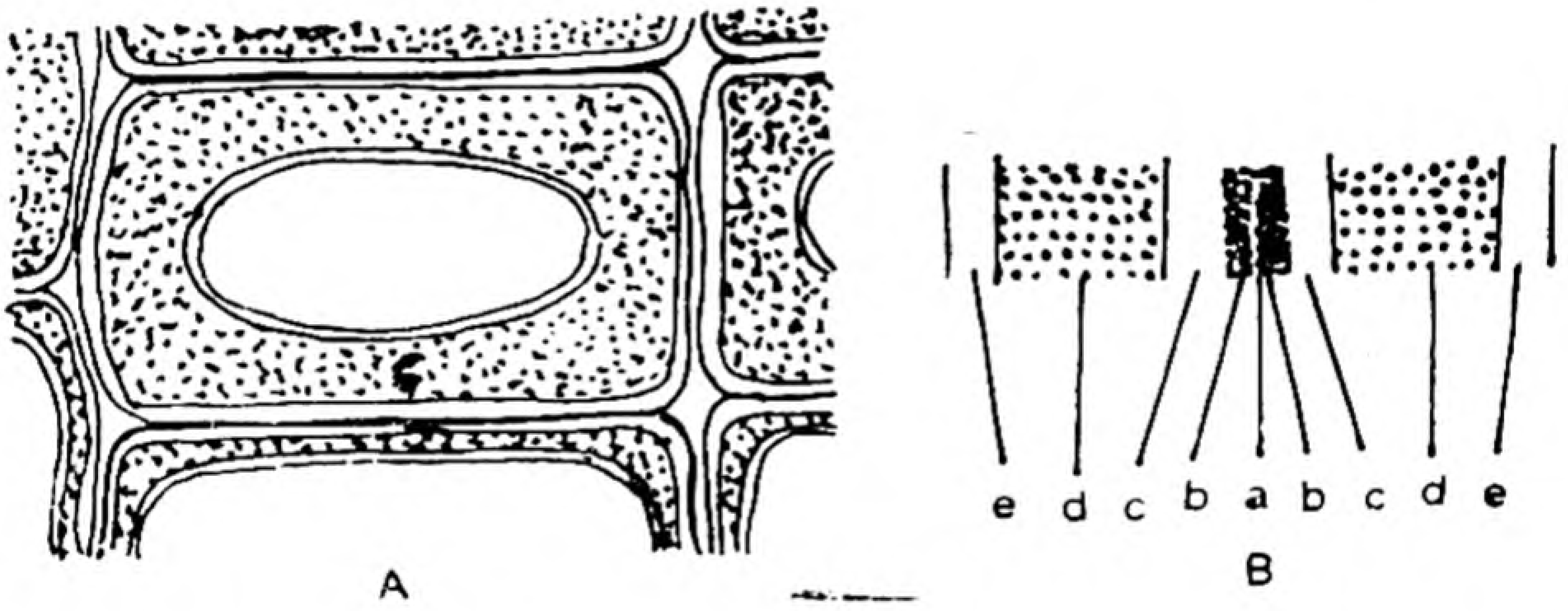


FIG. 2.7: Diagrams showing wall structure and middle lamella in thick-walled cells (wood fibres). A. cross section of one fibre and parts of seven adjacent fibres; B, cross section of adjacent walls and middle lamella more highly magnified. a, middle lamella; b, paimary wall; c, d, e, outer, median, and inner layers, respectively, of secondary wall. (After Bailey.)

The chemical and physical nature of all wall layers and of the middle lamella undergoes various changes as the walls mature, and may undergo even further changes at a later period, for example, during the transformation of sapwood into heart-wood. The middle lamella, at first containing much pectic material, becomes heavily lignified in thick-walled tissues and cutinized in epidermal regions. It shows no evidence of layering. The primary wall likewise becomes strongly lignified in wood and most sclerenchyma. Much of the mineral content of the cell wall is found here. In epidermal cells of seeds and fruits this wall is often gelatinous or mucilaginous. Lignification, cutinization, and suberization are the major changes in secondary walls. Gelatinous layers are frequent in the secondary wall of wood.

Method of Wall Building

The method of addition of building material as the wall increases in area and thickness is not wholly understood. Two processes have been described: *apposition*, the placing of new particles in layers on the surface of the earlier formed wall; and *intussusception*, the addition of new particles among those already in position. When the wall is considered a matrix to which new material is added throughout, intussusception must play a prominent part in increasing the extent and thickness of the wall. Particularly in the increase in area of parimary walls, intussusception must be the chief method. The layering of the secondary wall and the varying direction of the fibrils of its successive layers suggest that the addition of particles is periodically restricted to the layers next to the protoplast. Hence, considering the major layers alone, increase in thickness is by apposition; but each layer itself may be built by both apposition and intussusception. When the original matrix is increased by the addition internally of new micellae or fibrils, and when it is chemically transformed by replacement of particles or the deposition of new substances in the interstices of the meshwork, the method must be intussusception.

Thickenings of the Cell Wall

The secondary wall acquires different types of thickenings. This is specially seen in the cells which are to differentiate into tracheary elements *i.e.*, vessels and tracheids. In these cells the thickening is due to the deposition of lignin. The deposition of lignin is not uniform, hence we find different types of thickenings. Chiefly the following types of thickenings are seen in xylem.

Annular. Lignin deposition is in the form of annuli or rings. These rings are unconnected. The space between the rings is unthickened (Fig. 2.8 A).

Spiral. Lingnification occurs in the form of a spiral band (Fig. 2.8 B).

Scalariform (Ladder like). Wall thickening is in the form of transverse rods, very much resembling the rungs of a ladder (Fig. 2.8 C).

Reticulate. Lignin is deposited in the form of a network or reticulum. The unthickened areas of the cell wall are irregular in shape (Fig. 2.8 D).

Pitted. Lignin deposition is almost uniform over the wall, except for small pores here and there. These pores are called *pits* (Fig. 2.8 E. F).

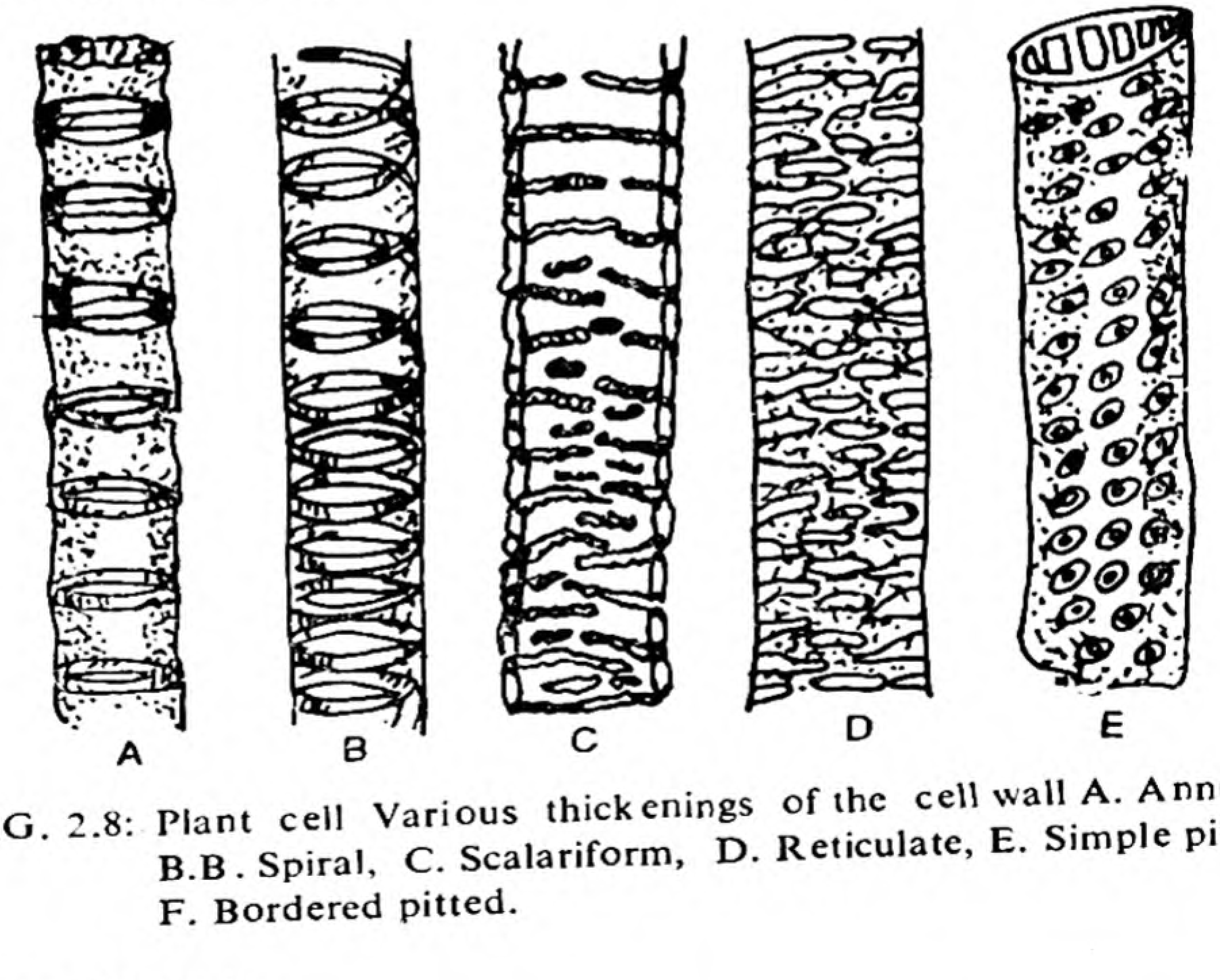


FIG. 2.8: Plant cell Various thickenings of the cell wall A. Annular, B.B. Spiral, C. Scalariform, D. Reticulate, E. Simple pitted, F. Bordered pitted.

Plasmodesmata

That the relation between cell wall and protoplast is very close is evident by the presence in the wall of many delicate threads of cytoplasm which connect the protoplasts of adjacent cells (Fig. 2.9). These threads, known as *plasmodesmata*, fill minute passages which constitute the only breaks in the primary walls of the two cells. They are characteristic of living cells and are undoubtedly present in all living parts of the higher plants, maintaining the continuity of the protoplasm of the plant although its units, the cells, are sharply set apart by firm walls. Their extreme delicacy and protoplasmic nature make special technique necessary for their demonstration in most tissues. When the fibrils of contiguous cells meet at the middle lamella there are often seen slight knob-like thickenings. These may be enlargements of the threads owing their form to the nature of the intercellular substance, which in early stages may be partly protoplasmic or they may be merely artifacts. Plasmodesmata are most readily seen in the endosperm of some seeds (*Diospyros*, *Aesculus*, *Phoenix*) where food storage has greatly increased wall thickness, and in some cotyledons. They areextremely fine in gymnosperms. They have not been demonstrated in the earliest stages of wall formation, and they disappear with the death of the cell. In such cells as tracheids and vessel elements even the channels in the wall in which they lie are apparently filled when they disappear with the protoplast.

They occur in groups or are scattered. Usually many of them are concentrated in restricted thin areas of the young wall, the primary pit-fields. In mature walls with secondary layers, the larger groups occur only in the pit membranes (the thin wall areas between two pits. It has been suggested that the presence of clustered plasmodesmata in the primary wall determines the position of a pit.

Origin. Plasmodesmata probably represent persisting connections of the protoplasts of daughter cells. Although their presence in the very delicate first walls has not been demonstrated, meristematic cells may show them well. In early stages of wall formation the wall is mesh-like, with pores and interstics which doubtless contain cytoplasm. The nature of the cell-plate fluid at this time is unknown but it is doubtless in part proto-

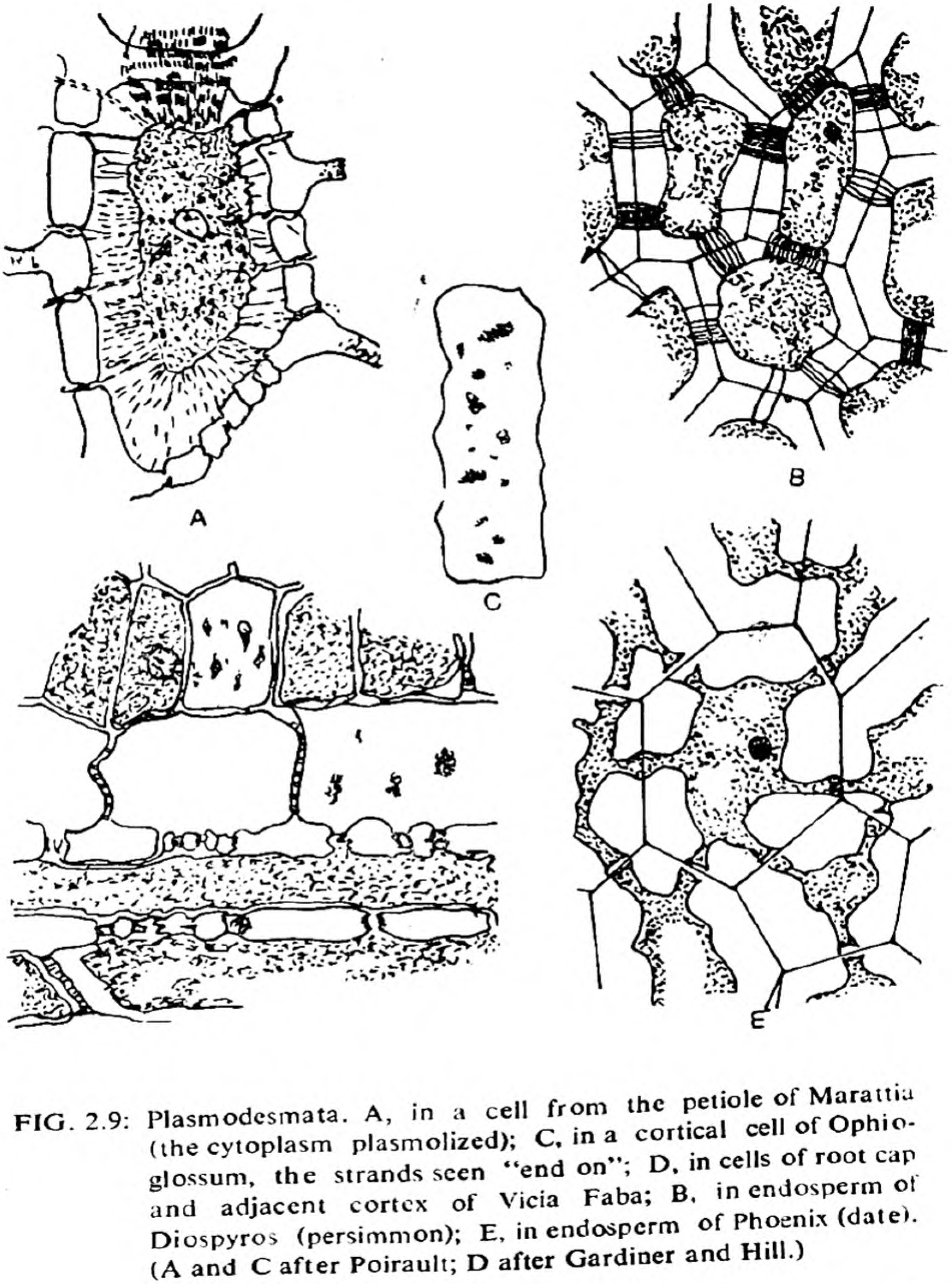


FIG. 2.9: Plasmodesmata. A, in a cell from the petiole of Marattia (the cytoplasm plasmolized); C, in a cortical cell of Ophioglossum, the strands seen "end on"; D, in cells of root cap and adjacent cortex of Vicia Faba; B, in endosperm of Diospyros (persimmon); E, in endosperm of Phoenix (date). (A and C after Poirault; D after Gardiner and Hill.)

plasmic. As cellulosic and pectic materials are laid down in the three layers between the two protoplasts the cytoplasmic connections are restricted to slender threads and at this stage become detectable.

As the wall increases in area with growth of the cell, the number of plasmodesmata seems to increase. The increase possibly results from the splitting of the original strands. New connections between protoplasts are apparently not freely made but when cells during growth establish new contacts, secondary plasmodesmata may form in these areas. During symplastic growth the strands ase distributed over larger areas, accompanied perhaps by multiplication. In intrussive growth they are ruptured and when the new-contact areas of both walls are young enough are replaced by new strands. It has been claimed that no secondary strands are formed; that evidence for this is seen, for example, in their absence on the new-contact walls of wood fibres and developing vessel elements. But where such new contacts of enlarging vessels are with parenchyma cells new plasmodesmata are formed. The presence of plasmodesmata in graft hybrids and periclinal chimaeras between cells of different genetic origin seems to be sufficient evidence that plasmodesmata may develop secondarily.

Function. It has been suggested that plasmodesmata are "channels of translocation of solids," that they "conduct stimuli," but no demonstration of such functions has been made. Their existence is doubtless important in many ways because they unite the otherwise isolated protoplasts of the plant body into a single protoplasmic structure.

SCULPTURE AND MODIFICATION OF THE WALL

Pits

During early stages in the thickening of the primary cell wall, small sharply defined areas in the primary pit-fields are left thin and uncovered. As thickening continues, recesses or cavities are left in the wall at these places. These cavities, together with the walls that surround them—the exposed area of primary wall that forms the "bottom" or external wall, and the side walls and roof—are known as *pits*. (The term pit is often applied to the cavity alone but best covers not only this but the modified wall area in which the pit lies.) Pits are conspicuous features of mature cell-wall structure. They vary in type, in form, and in abundance. They are characteristic of all kinds of cells and are probably lacking only in cells with very thin walls. Functionally. pits seem to be diffusion areas, regions where better interchange between cells may occur. That pits may be areas of interchange of another type also is apparent from the fact that in living cells groups of plasmodesmata are frequently or always present in the pits in larger numbers than elsewhere (Fig. 2.9).

Pits are structural features either of the primary wall alone or of the primary plus the secondary wall. Those that involve both wall layers are more readily seen; those of the primary wall alone are conspicuous only where the wall is fairly thick.

Pit-Pairs

A pit has a complementary or opposing pit in the wall of the contiguous cell. The two pits form a structural and functional unit, a *pit-pair*. (The use of the term "pit" for both the pit and the pit-pair has long caused difficulty and confusion in descriptions. The adoption of "pit-pair" for the double unit corrects the morphological error.)

The recess of the pit is the *pit cavity*; the membrane that separates the two cavities of a pit-pair is the *pit membrane*, or *closing membrane* of the pit-pair; the opening or mouth of the pit at the inner wall surface is the *pit aperture*. A pit-pair has two cavities, two apertures, and one closing membrane. The closing membrane consists of three layers—the primary wall of each of the two cells (differene in thickness and chemical nature from adjacent parts of the primary walls) and, between these, the middle lamella which also is different from its adjacent parts in this area.

Two types of pits are recognized: *simple* and *bordered*

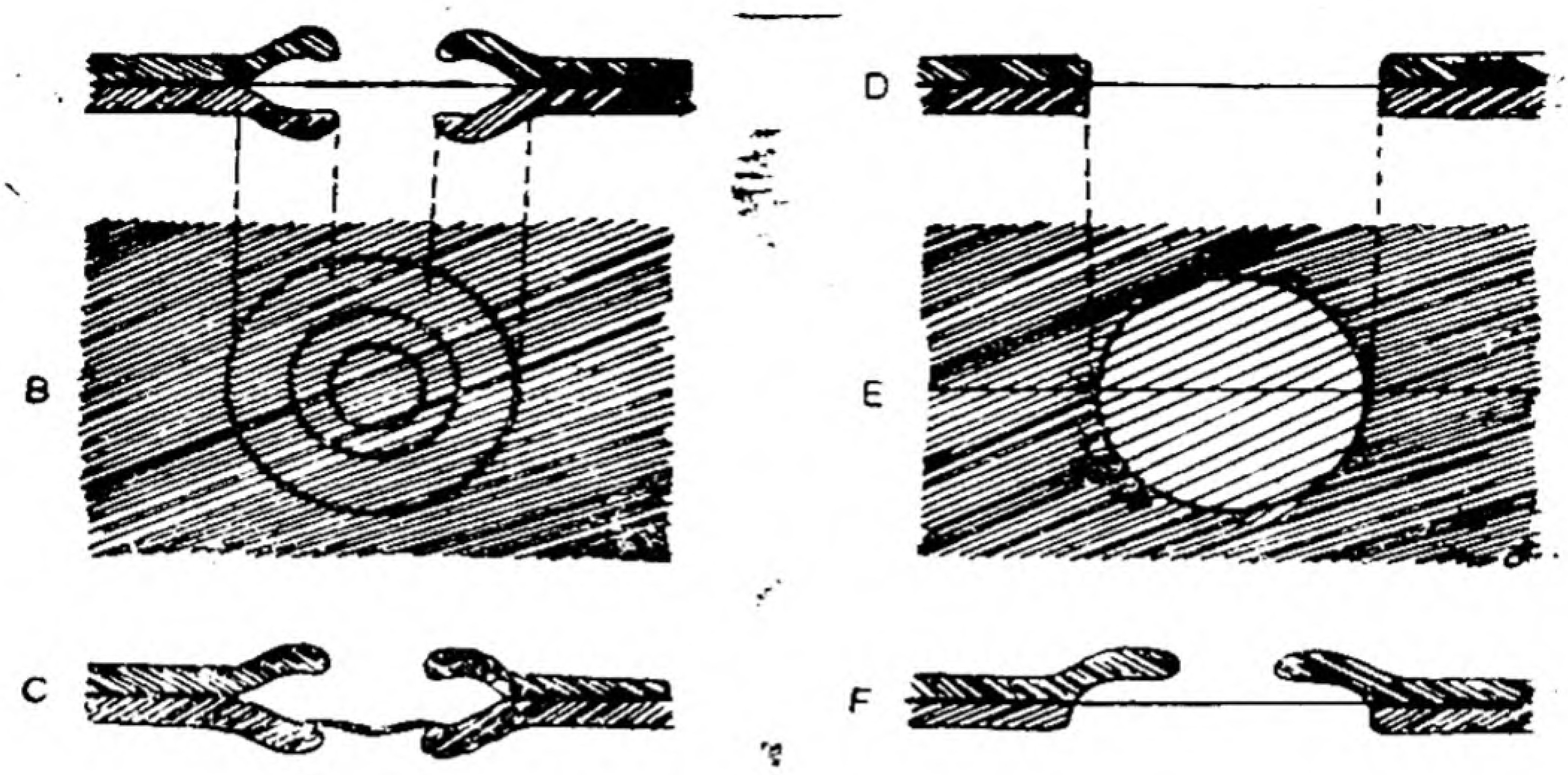


FIG. 2.10: Diagrams of three kinds of pit-pairs. A, B, section and face view of bordered pit-pair, showing the overarching borders of secondary wall which enclose the pit cavities, between which passes the closing membrane whose central portion is thickened to form the torus; C, section of same showing the closing membrane in a lateral position, the torus closely appressed to the pit aperture; D, E, section and face view of a simple pit-pair in which the secondary wall does not overhang the pit cavities and the closing membrane has no torus; F, section of half-bordered pit-pair, the secondary wall overarching only one pit cavity, no torus. (A-C, from Sharp, after Bailey).

(Fig. 2.10). These form *simple pit-pairs* when both members of the pair are simple; ***bordered pit-pairs*** when both are bordered; and *half-bordered pit-pairs* when one pit is simple and the other bordered.

Simple pits are those in which, as the wall is built up in thickness, the cavity remains of the same diameter, or is enlarged, or is only slightly constricted, and the closing membrane is simple is form and structure. In section, such pits show the side walls of the pit at right angles to the primary wall, sloping away from the margin of the exposed closing membrane, or sloping only slightly and gradually toward the center (Fig. 2.11). Simple pits with chambers slightly narrowing to the aperture somewhat resemble some forms of bordered pits—showing evidence of a narrow border in face view—but the closing membrane is without a torus.

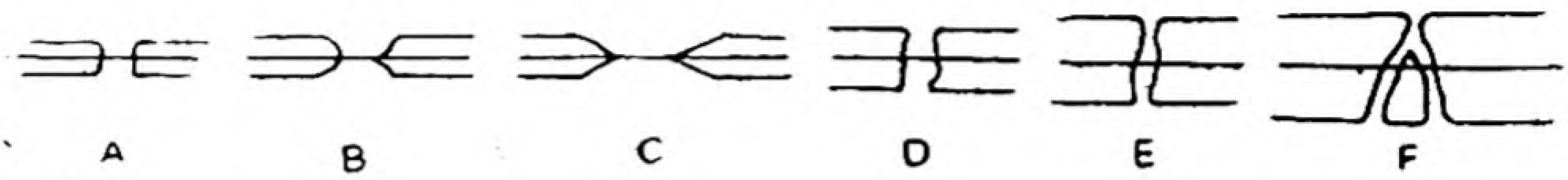


FIG. 2.11: Diagrams showing varieties of simple pit.pair stracture as seen in sectiou. A, the walls of the cavities at right angles to the closing membrane; B, C, the walls sloping away from the closing membrane; D, E, the cavities narrowing slightly to the apertures; F, two pit-pairs, with two pits fused to form one cavity at one aperture. In no form is the pit cavity differentinted into cavity and canal as in bordered pits.

In *bordered pits*, the cavity, as the wall thickens, is so constricted that a rim or roof is built, overarching the recess. The closing membrane is elaborate in form and structure. The pit is called "bordered" because in face view the rim forms a border around the aperture. In section such pits show the cavity more or less nearly enclosed by the secondary wall. Where the secondary wall is thick. (Fig. 2.12), the pit cavity is divided into parts, an external *pit chamber*, and a passageway leading from the lumen to the chamber, the *pit canal*; where the secondary wall is thin (Fig. 2.10), no distinction of chamber and canal can be made. The pit canal in bordered pits has an *outer aperture*, the opening into the pit chamber, and an *inner aperture*, the opening into the cell lumen. The cavity of a simple pit is not divided into chamber and canal, but deep simple pits in thick walls have "*canal-like cavities*." Such a pit has only one aperture.

In *half-bordered pit-pairs* both pits vary type as do the pits of simple and bordered pairs. The closing membrane may have a weak torus or no torus.

The bordered pit-pair is complex not only in the form of its cavity but in the structure and behaviour of its membrane. The membrane of a bordered pit-pair (consisting of parts of

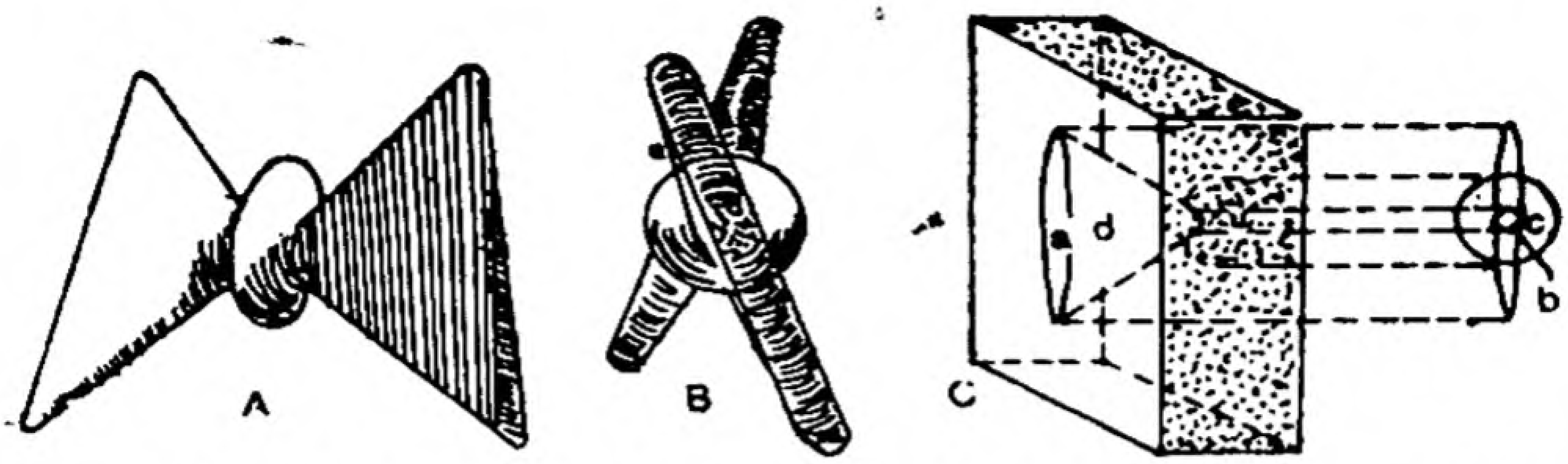


FIG. 2.12: Diagrams showing bordered-pit structure of the type shown in Fig. 29A, 4. A, B, showing the form of the cavities of a pit-pair—the inner apertures are slit-like; the small chambers, flat-dome shape; the canals like flattened funnels. C, a pit shown in position in a thick cell wall. a, the inner aperture; b, the outer aperture; c, the chamber; d, the canal. (C, after Record, modified.)

two primary walls and the middle lamella) has a thickened central part, the *torus*, surrounded by a delicate marginal area. The triple nature of this membrane is not readily evident because all the layers are much alike chemically (mainly cellulosic) and structurally. In some of the gymnosperms the thin area of the closing membrane is perforated by minute openings or even by larger pores, so that the torus is suspended by a meshwork or, rarely, by a few stra like pieces. The existence of these perforations has been clearly demonstrated by the passage of minute solid particles (finely ground India ink) from tracheid to tracheid.

The closing membrane of many bordered pit-pairs may change position within the cavities, apparently because of changes of pressure within the cells. The torus may occupy the median position (Fig. 2.10A); or lie close against the aperture on either side (Figs. 2.10 C, B). When in the lateral position it may be distorted by the forcing of its central part into, or even partly through, the aperture. The diameter of the torus is always greater than that of the aperture so that, when the torus is in the lateral position, passage through the pit-pair can be only by diffusion through the torus. With the torus in the median position, the thinner margin provides passage of a perhaps different type. In the conifers with actual perforations, free passage is possible when the torus is in median position, but this has not been demonstrated in other groups. Membranes capable of these changes of position are characteristic of living spring wood. Those of summer wood are inflexible (in some woods 20 to 60 per cent are said to retain median position) and all membranes of heartwood are "frozen" in position by the changes incident to heartwood formation. These pit-membrane characteristics are important from he standpoint of impregnation of wood under pressure with preservatives and other substances. Under pressure the membranes of heartwood and those of the summer layers of sapwood, held rigidly in position (perhaps in heartwood often cracked by shrinkage), can be ruptured to permit the passage of fluids; those of living spring wood are forced into the lateral position with the torus jammed into the aperture and cannot be ruptured.

The simple pit-pair has no torus; the half-bordered pit-pair has either no torus or a weakly developed one. The closing membrane of simple pit-pairs retains its median position; that of half-bordered pit-pairs may bulge well into the lumen on either side when the apertures are large.

Departures from normal pit-pair structure are few. A large pit in one cell may have two or more smaller pits opposite it in the adjacent cell. This is *unilateral compound pitting*. A pit without a complementary pit is a *blind pit*. Such pits are possibly halves of pit-pairs that have been split apart in the readjustment of cell contacts during the cell growth. (The pits without complementory pits commonly seen in sections of wood cells and other thick-walled cells are halves of pit-pairs that have been cut obliquely in sectioning).

Simple pit-pairs, with the canals slightly and gradually narrowing toward the aperture, are frequent in thick-walled parenchyma, in some sclereids, and elsewhere. Some of these have been incorrectly called "bordered." These do not have the pit membranes of bordered pits or the distinction of cavity into chambers and canals. The bordered pit, as a morphological structure, is characteristic of and restricted to water-conducting cells and their phylogenetic derivatives. If pits with slightly overhanging borders are called "bordered", important morphological structure is ignored.

Simple pits occur in walls that are wholly primary and those that are both primary and secondary; bordered pits occur only in primary plus secondary walls. Simple pits occur in many kinds of cells; bordered pits are characteristic of water conducting cells and of kinds of cells morphologically derived from these cells. In a given cells or kind of cells, the type is constant.

Variations in Size and Structure of the Bordered Pit

The bordered pit-pair varies greatly in size and structure (Figs, 2.13, 2.14, 2.15). The prominent modifications of its typical form and size are those associated with the specialization and reduction of the pit as it occurs in fibre tracheids and fibres. In the typical bordered pit-pair, the cavities, which are circular in outline, are large, and the apertures are also circular (Fig. 2.16A). Where such pit-pairs occur in thin walls. the borders may arch and bulge into the lumen, as in the spring-wood tracheids of *Larix* and species of *Pinus* (Figs. 2.15E, 2.16I). These pit-pairs are large in comparison wit the cells to which they belong and, in section, may resemble small cells (Fig. 2.15E). Where the wall is thicker the presence of a pit causes no mounding or bulging of the wall, and the aperture opens into the pit canal, not directly into the chamber.

In general, the thicker the wall in bordered pit-pairs, the smaller the chamber and the smaller the aperture. In thick walls, although the chambers and outer apertures remain always circular in outline, the inner apertures become narrowed and elongated, being elliptical to slit-like in outline (Fig 2.16). When only slightly narrowed, the inner aperture, seen in face view, may extend only part way to the margin of the cavity (Fig. 2.16A 2), or may reach the very limit of the cavity Fig. 2.16A 3), so that there are no borders at those points.

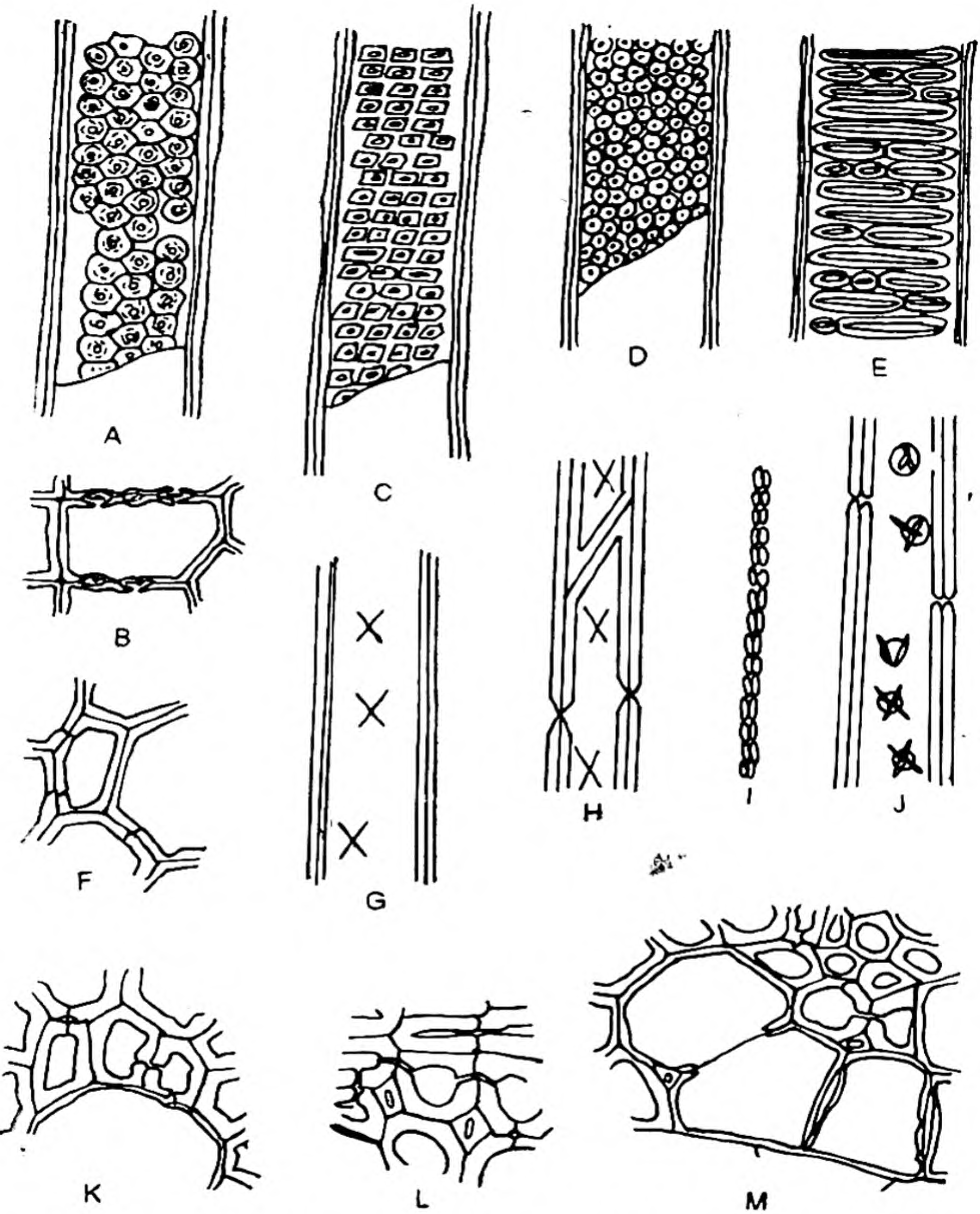


FIG. 2.13: Bordered pit-pairs, A, B, crowded angular pit-pairs in face view and in section in tracheid of Agathis australis; C, rectangular pit-pairs, face view, in horizontal rows in vessel of Liriodentron; D, crowded pit-pairs, face view, in spiral rows in vessel of Acer rubrum; E, elongate pit-pairs in face view in vessel of Magnolia acuminata—the same pit-pairs in vertical section in I, and in transverse section in M; F,

G, pit-pairs with narrow, inner apertures, in section and face view in thick walls of wood fibre of Pepulus tremuloides; H. similar pit-pairs in wood fibres of Magnolia, face view and longitudinal section—the same pit-pairs in transverse section in L; J. K, pit-pairs with narrow, unsymmetrically placed inner apertures in wood fibre of Malus pumila in face view and in longitudinal and transverse section; M, large, elongate pit-pairs (E) in vessels and small pit-pairs in fibres, both in transverse section in Magnolia.

Where the inner aperture is much narrowed, the slit usually extends far beyond the limit of the chamber as seen in face view (Fig. 2.16A, 4—6). In reduction of pit size in thick walls, the greater the reduction in diameter of the chamber, the greater the increase in length of the inner aperture. These much elongated apertures occur only in thick walls, and hence the pit canal through the thick wall to the small inner chamber is in shape like a much flattened, or laterally compressed, funnel (Fig. 2.12). The outer aperture of this funnel-like canal remains circular. Where the inner pit apertures are circular, the two apertures of a pit-pair lie directly opposite one another (Fig. 2.16), coinciding in position; where the inner apertures are elongate, the appertures are commonly crossed, usually symmetrically (Fig. 2.16A 2—6). (The angles at which the inner apertures lie are apparently controlled by the orientation of the strands of fibrils that make up the major layer of the secondary wall.). These crossed pit apertures are conspicuous features of pit marking in the walls of wood cells (Figs. 2.13, H, J, 2.14A). The pit cavity in such pits is complicated in form (Figs. 2.12 and 2.16). With extreme reduction the borders of the pits nearly disappear, and slit-like pit-pairs, essentially simple in structure, are formed (Fig. 2.16A 6). Morphologically, such pit-pairs are, however, clearly bordered pit-pairs. The pits of libriform wood fibres are often wholly vestigial being simple and hardly more than markings on the wall. In much reduced bordered pit-pairs the closing membrane is of simpler structure, with no torus and no capacity to change position. Such pits are probably physiologically functionless or nearly so.

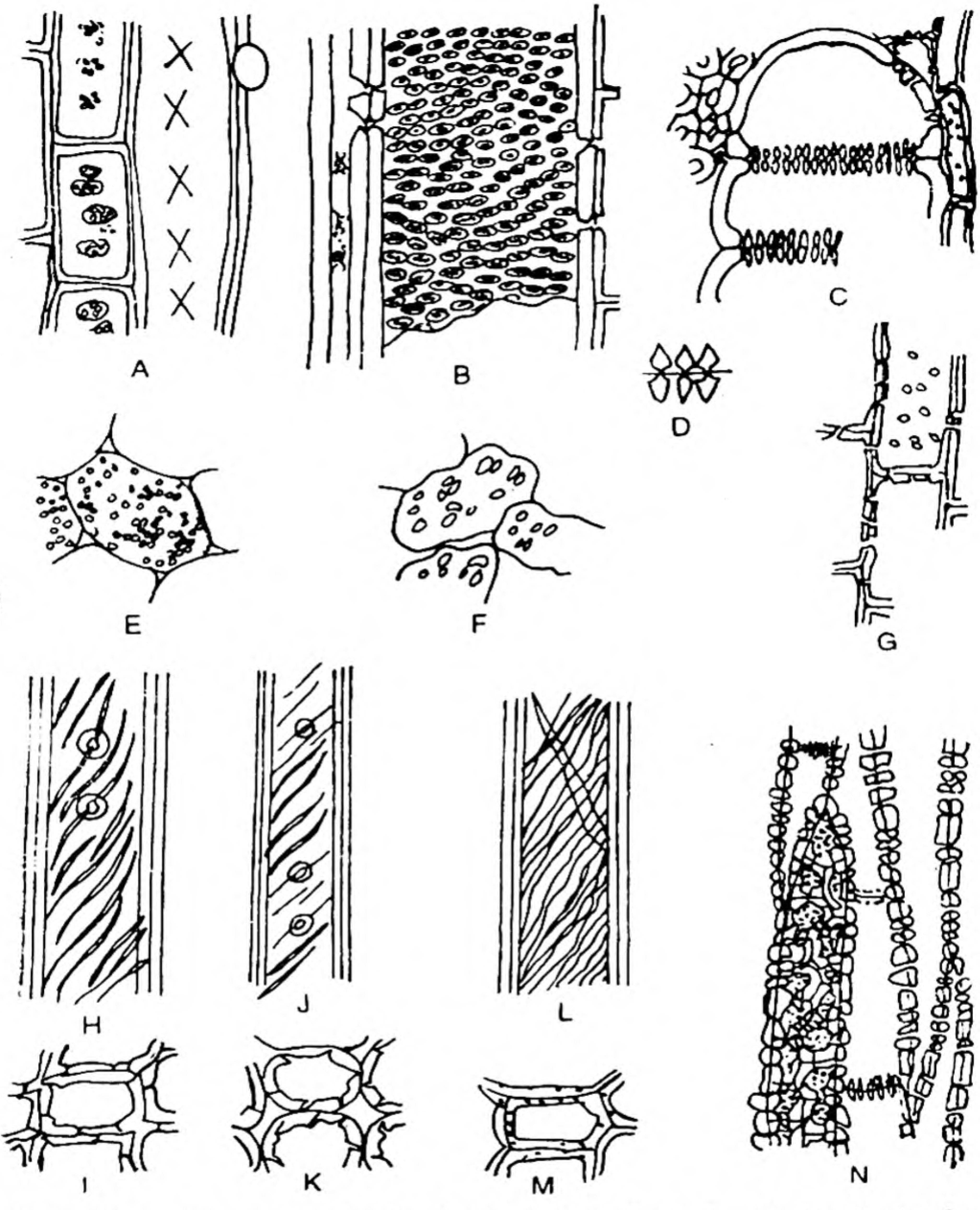


FIG. 2.14: Pit-pairs and other [wall sculpturing. A, clustered simple pit-pairs in wood parenchyma, and very small bordered pit-pairs in fibres, face view, in Fraxinus Americana; B, bordered pit-pairs of two-forms, face view, in vessel and in fibre, and half-bordered pit-pairs, in section, between vessel and parenchyma cell, in Diospyros (ebohy); C, the same pit-pairs as in B, in transverse section, also simple pit-pairs, face view and section in ray and parenchyma

cells; D, detail section of bordered pit-pairs between vessels, as shown in C; E and F, simple pit-pairs in thin-walled parenchyma cells—E, in pith of Chenopodium, F, in pericarp of Citrullus; G. simple pit-pairs in thick-walled parenchyma of pith of Clematis; H and I, J and K, microscopic checking, face view and section, of secondary wall in tracheids of Pinus Strobus and Sequoia sempervirens—the cracking extending through pit apertures simulates elongate apertures: L, M, erosion of secondary wall by fungus hyphae, simulating checking, face view and section, in tracheid of Podocarpus; N, simple pit-pairs in thick-walled ray cells and wood parenchyma, face view and section, in late summer wood of Magnolia.

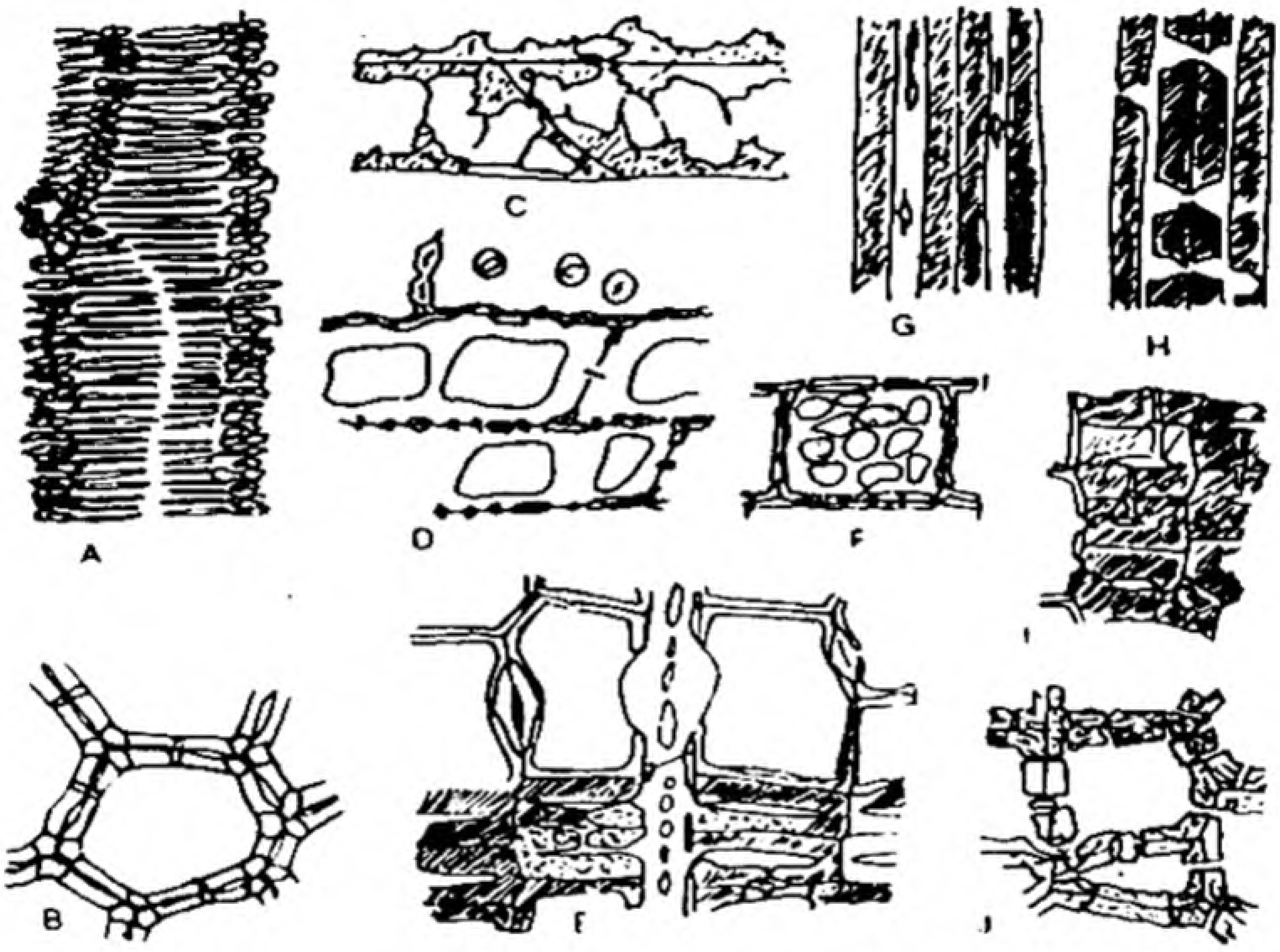


FIG. 2.15: Pit-pairs, A, B, elongate bordered pit-pairs of tracheid of Osmunda Claytoniana, face view and section; C, bordered pit-pairs and "dentations" of wall in section in marginal ray cell of Pinus Banksiana; D, full-bordered, half-bordered, and simple pit-pairs, face view and section, in wood ray cells, radial section of Pinus Strobus; E, similar, in part the same, pit-pairs, in face view and section, in tracheids and ray cells in transverse section of wood of Pinus Strobus; F, simple pit-pairs in section and half-bordered pit-pairs in face view in ray cells of wood of Salix alba; G, H, and I, small bordered pit-pairs with narrow, vertical inner apertures coinciding in position, and long, narrow pit-canals between pit chamber and lumen, face view, longitudinal section, and transverse section, respectively, in wood fibres of Eucalyptus globulus; J. simple pit-pairs in stone cells of phloem of Platanus.

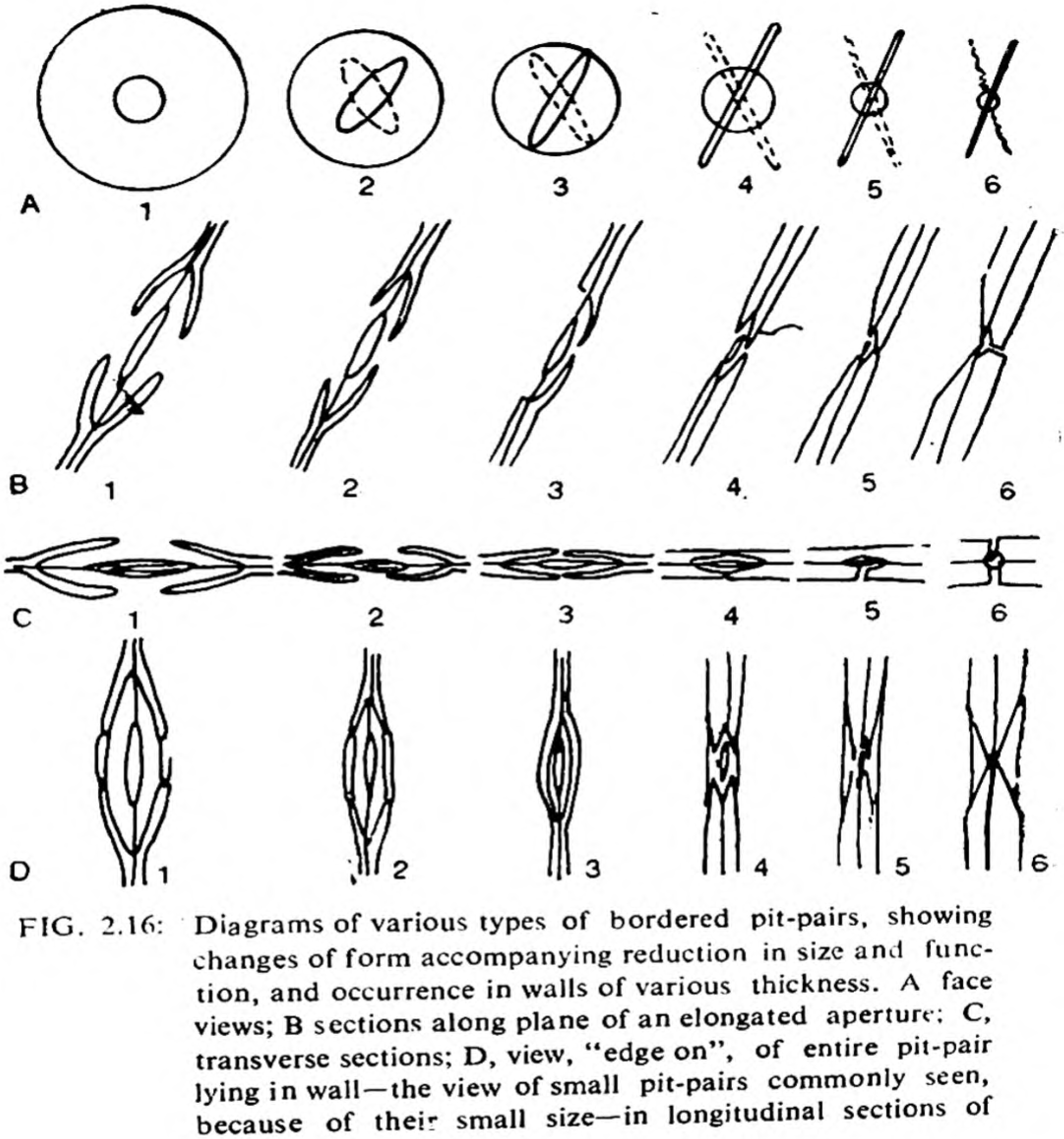


FIG. 2.16: Diagrams of various types of bordered pit-pairs, showing changes of form accompanying reduction in size and function, and occurrence in walls of various thickness. A face views; B sections along plane of an elongated aperture; C, transverse sections; D, view, "edge on", of entire pit-pair lying in wall—the view of small pit-pairs commonly seen, because of their small size—in longitudinal sections of wood.

Vestured Pits

Pits that have minute outgrowths from tde surface of the side wall of the pit chamber or along the rims of the apertures are known as *vestured pits*. The projection that form the vesture are of many types—filamentous, papillose, coralloid; branched and anastomosing, or simple. They may be scattered or closely clothe the surface of the wall. In some pits they cover also the surface of the wall of the vessel in which the pits occur. When long, they may fill the pit cavity and project into the cell lumen. Only bordered pits are vestured; in half-bordered pit-pairs the simple pit is without vesture.

The refractive and deep-staining qualities of these minute structures give a punctate or recticulate appearance to pits seen in face view. For this reason such pits were formerly called "cribriform" and "sieve-like" in the mistaken interpretation that the closing membrane was perforated like a sieve plate.

Vestured pits are probably restricted to the tracheary elements of the secondary wood of certain angiosperm families, for example the Leguninosas. Cruciferae, Myrtaceae, Caprifoliaceae.

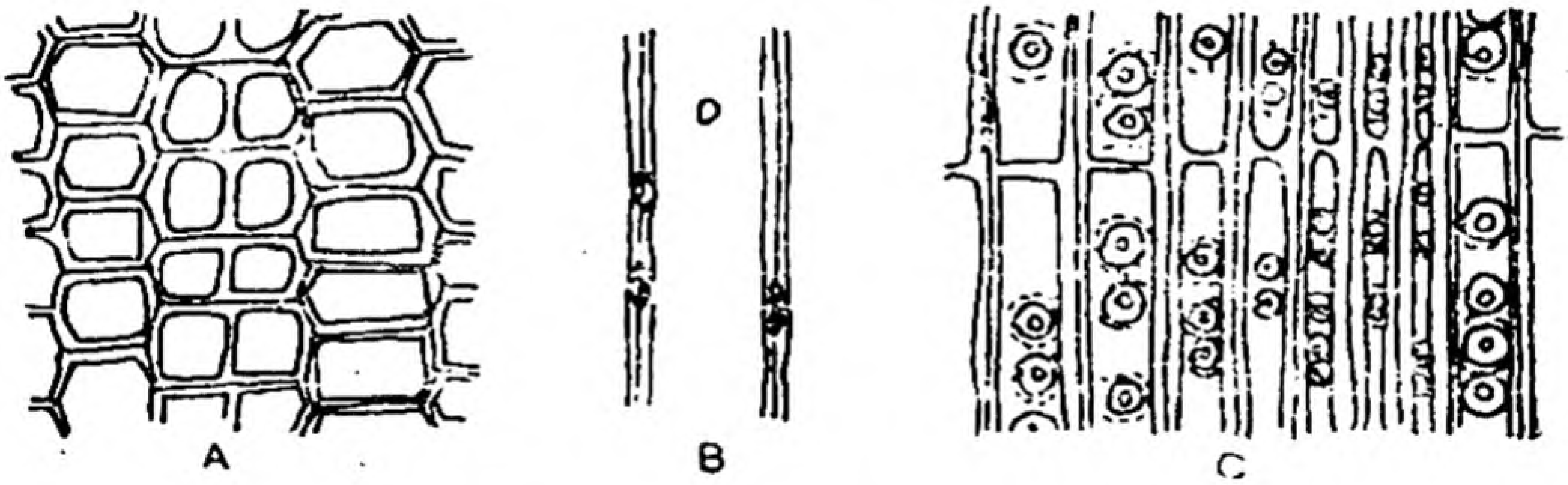


FIG. 2.17: Trabeculae in the tracheids of Abies, balsamea. A transverse section; B tangential section; C, radial section.

When present, they are constant throughout the tissue in the species or genus and probably also in subfamilies, and families and so may be important in systematic and phylogenetic studies. No function has been suggested for them. Then presence is associated with highly specialized wood types and they appear to indicate a highly specialized pit type.

Other Wall Sculpturing

The modelling of the inner surface of the cell wall is in large measure due to the presence of pits. Pits and perforations of vessel walls form the conspicuous structural modifications. Also prominent are the secondary-wall thickenings of protoxylem—rings, spirals, and reticulations. Other internal thicknings or projections occur—the "dentations", really ridges, of the ray tracheids of hari pines (Fig. 2.15C); the Casparian strips of some endodermal cells; the trabeculae of conifer tracheids (Fig. 2.17); the supporting bars or meshes of cells of the velamen and anther wall.

Trabeculae. Rod-like or spool-shaped wall projections crossing the lumen radially are known as *trabeculae*. They are frequent in the secondary wood of conifers, rare elsewhere. They usually occur in rows radially, and the rows may extend across the cambium and into the phloem.

Crassulae

Structural variations within the wall itself may appear like surface features. Bar-like or crescent-shaped thickenings of the primary wall and middle lamella that separate or partly encircle individual bordered pits or small groups of pits may be visible through the secondary wall when properly stained. These *crassulae* are the persisting marginal rims of the primary pitfields of the young cell wall. The secondary wall covers but does not form a projection over these primary ridges so that they are not features of the sculpture of the wall surface. The names "bars of Sanio" and "rims of Sanio" have long been applied to crassulae but these terms have been confused in usage and in part applied also to trabeculae. The new term "crassulae" should replace both older ones.

Centrifugal Thickening

In cells that have free surfaces the wall is apparently sometimes thickened centrifugally. Some hairs and other epidermal cells, some sclereids, and certain cells abutting on large intercellular spaces have minute nodules, granules, or ridges on the outside of the wall. Some of these are parts of the wall; others are deposits on the surface, as is the cuticle. The external wall layers and surface projections of spores and pollen grains are formed in part by tapetal fluid or mother-cell cytoplasm.

THICKENING AND ALTERATION OF THE CELL-WALL

We have already seen that in the young cell the cell-wall is thin and composed mainly of cellulose deposited on a middle lamella, probably consisting of calcium pectate. As the cell increases in size, usually by vacuolation, fresh cellulose, built up by the activity of the living protoplasm, may be incorporated in the cell-wall in two ways: *intussusception*, where the new cellulose particles are deposited between those of the existing cell-wall so that the latter grows in are; and *apposition*, in which the cellulose particles are laid down on the surface of the original wall, by which msans the latter is thickned. It is probable that both methods may go on simultaneously, the former predominating during active enlargement of the cell, the latter when the cell-wall is increasing in thickness.

The thickened cell wall will have the same composition as the young cell-wall, and will possess the same or very similar physical and chemical properties, but often, besides being thickened, the cell-wall becomes altered by impregnation with substances other than cellulose. It may be cutinised, lignified become mucilaginous, or be impregnated with mineral matter such as silica.

Hemicelluloses

On the original cellulose cell-wall we may get deposited hemicelluloses. Whereas cellulose is built up from glucose molecules, hemicellulose (sometimes called reserve cellulose) is built up from glucose and the molecules of other sugars such as fructose, galactose and mannose. Hemicelluloses are deposited on the cell-walls especially in some seeds, *e.g.*, nux vomica, lupin, date and coffee, where they represent reserve food-materials which will be used when the seed germinates. During the growth of such cell-walls the cytoplasm maintains intimate contact with the suaface of the cell-wall, and also through its thickness, by means of delicate threads, which from protoplasmic connexions between contiguous cells. These *plasmodesma* can be demonstrated *e.g.*, in the collenchyma of cherry-laurel leaves, in the thick-walled cell of the endosperm of nux vomica, where they are more or less evenly distributed, and of date-stones, where they are limited to pits.

Cuticularisation

Cuticularisation is due to the formation of a waxy substance called cutin, which forms a continuous layer, called the cuticle, over the external walls of epidermal cells. The cuticle is probably secreted through the epidermal cell-well by the activity of the living protoplasm. It is almost impermeable to water, and hence helps to protect the cells below it from excessive loss of water. It also gives firmness to the cell-walls. The cuticle varies in thickness, being thicker in plants which grow in dry, sunny situations; its surface may be smooth, giving the glossy appearance to leaves, as in many evergreens, and to fruits, such as apples and tomatoes, or it may be slightly ridged, appearing finely striated under the microscope, as in leaves of *Atropa belladonna.*

Cutin may also impregnate the cell-wall, in which case it may affect the outer walls only, but, sometimes also the lateral walls, of the epidermis.

Wax

Covering the cuticle there may by layer wax, giving a matt or glaucous surface, *e.g.*, to leaves, and forming the "bloom" of fruits such as grapes and plums. Occasionally the wax layer is quite thick, as on the leaves of the wax palm (*Copernicia*), and can be utilised commercially as vegetable wax.

Suberisation

This is due to the formation of a fat-like substance called suberin. Cork cells have suberised walls. It is the middle layer of the cell-wall which is suberised, and thus rendered almost impermeable to water.

Cutin and suberin are stained yellow by iodine solution, and yellow or brown by Schulze's solution. They are not acted on by sulphuric acid.

Lignification

Lignification is due to the deposition in the cell-wall of a number of substances collectively known as lignin, which become intimately mixed with both the middle lamella and cellulose layers of the cell-wall. The mode of origin of the lignin is obscure, but lignification of the cell-wall is usually accompanied, or followed, by the death of the protoplasm in the cell. Hence cells with lignified walls are generally dead. Lignification confers increased strength and rigidity on the cell-wall, and at the same time reduces its elasticity (Fig. 2.18). It leads to profound changes in the chemical reactions of the wall which, if impregnated with lignin, is stained a bright yellow with aniline sulphate or chloride, yellow or brown with iodine, yellow with Schulze's solution, and bright red with phloroglucin and hydrochloric acid.

Delignification can be accomplished by prolonged boiling of lignified tissues *e.g.*, wood, in solutions of calcium bisulphite or caustic soda under pressure, or by alternate treatment with chlorine and sodium hydroxide. The cellulose will remains. Cellulose wadding is delignified gymnospermous wood.

The Cuticle

The surface of the aerial parts of vascular plants is covered by a layer of cutin, the *cuticle*. The cuticle covers the epidermis closely, following the contour of all cells (Fig. 2.19). Cutin is impermeable to water and the cuticle contributes to the control of water loss from the underlying cells. On young parts the cuticle is thin and pliable; as growth continues, it is stretched and added to in thickness. When growth of the tissues beneath

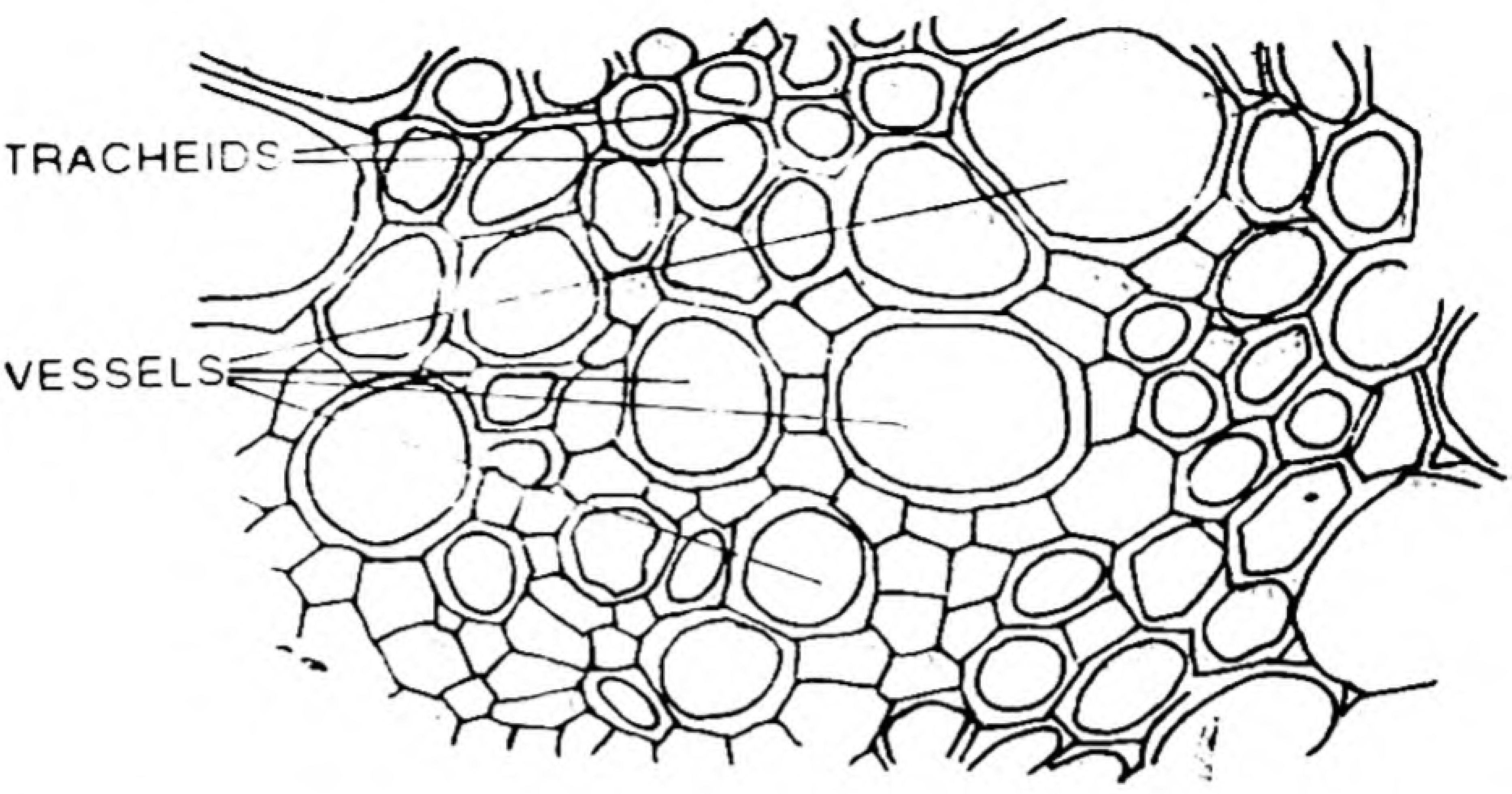


FIG. 2.18: Lignified Elements of the Xylem as seen in Transverse Section.

is complete, the cuticle becomes firm and highly resistant to stretching and to breakdown by any agent. (Cuticles persist, little changed, even in coal.) In thickness and in character, the mature layer varies greatly it is thin in plants of shade and moist habitats, generally thick in those of dry and sunny situations. Its thickness on the leaves and fruit of the same plant vaires somewhat with poition on the plant and from season to season with whether conditions. On ephemeral organs and aquatic plants it remains very thin and may not be readily detectable. Typical roots have no cuticle.

The only breaks in the continuity of the cuticle are the openings of stomata and lenticels. The cuticle extends for varying distances into the stomatal apertures (Fig. 2.20A) and a thin layer of cutin may coat the stomatal chamber. The walls of large air chambers may have a thin cuticle, and small intercellular spaces between the epidermis and mesophyll cells may also be coated with cutin.

In origin, the cuticle is a secretion of the epidermal cells and perhapy also of underlying cells. The cutin, in apparently a liquid or semiliquid from, is passed through the outer wall in

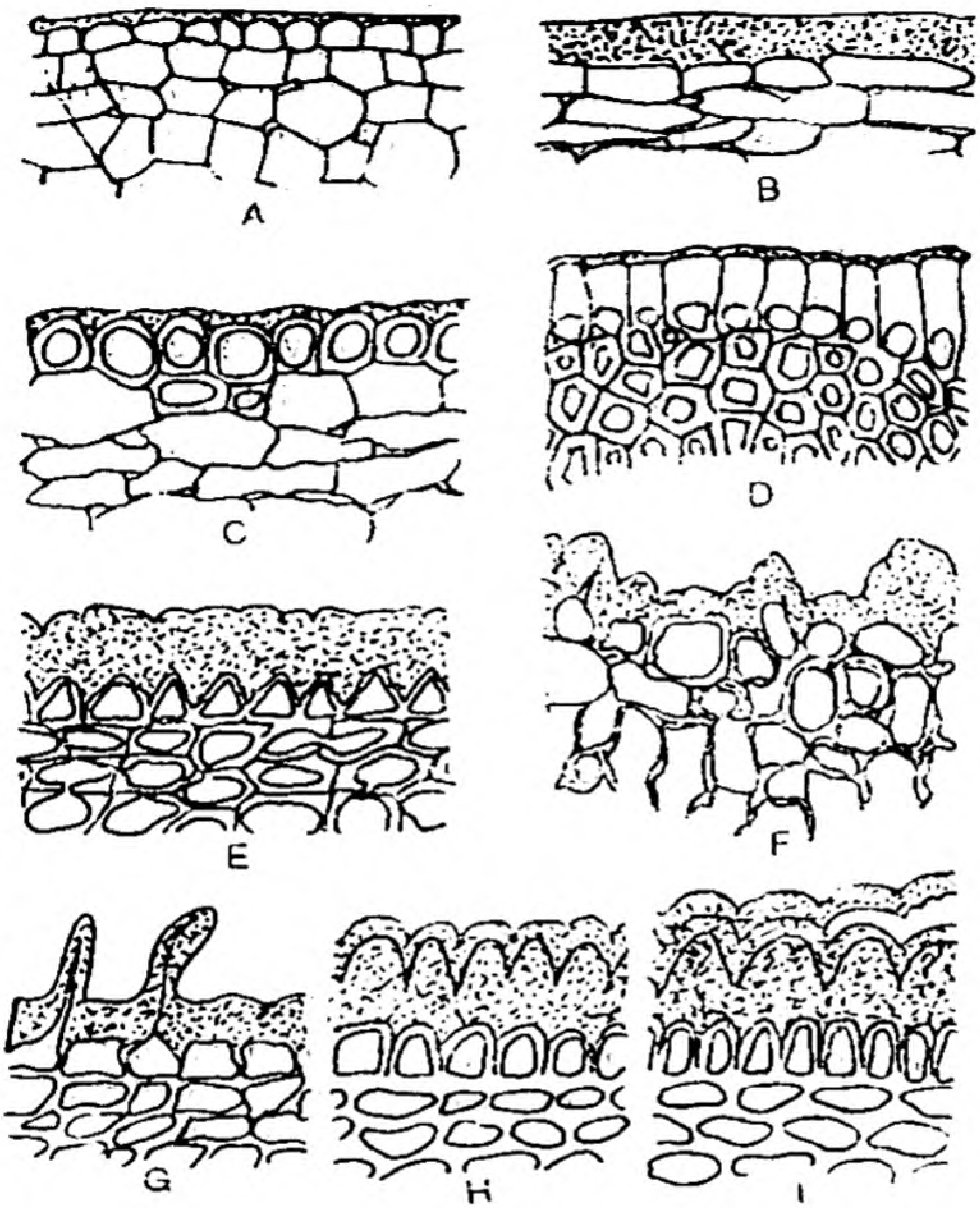


FIG. 2.19: The cuticle. Sections of the epidermis and adjacent tissues, showing various degrees of thickness and of extent of the cuticle between epidermal cells. A Citrus sinensis (orange), fruit; B, Malus pumila (var. Ben Davis), fruit; C, Dracaena Goldieana, stem: D, Dasylirion serratifolium, leaf; E, Acer pennsylvanicum, stem, the older, outer layer cracking and disintegrating; F, Smilax rotundifolia, stem; G. Vaccinium corymbosum, stem; H and I, Coanus circinata, stem, showing two stages, the outer, alder layers being replaced by new ones formed below.

an unknown manner. On the surface it forms a continuous layer and becomes hard and tough. According to one view, droplets of cutin pass through exceedingly minute openings of the outer wall; according to another, they are formed in the deeper lying cells and migrate along the walls, reaching the surface through the anticlinal walls of the epidermis. It is clear that the cuticle is thickened from below, although in early stages—and perhaps at some other times—new material is apparently dissolved in the preexistin layer. An example of the manner in which the cuticle is repaired or add to is seen in young steams and leaves which in early stages are clothed with hairs that are later shed; the spaces left in the epidermis by the bases of the hairs—now breaks in the cuticle—are filled by deposits of cutin.

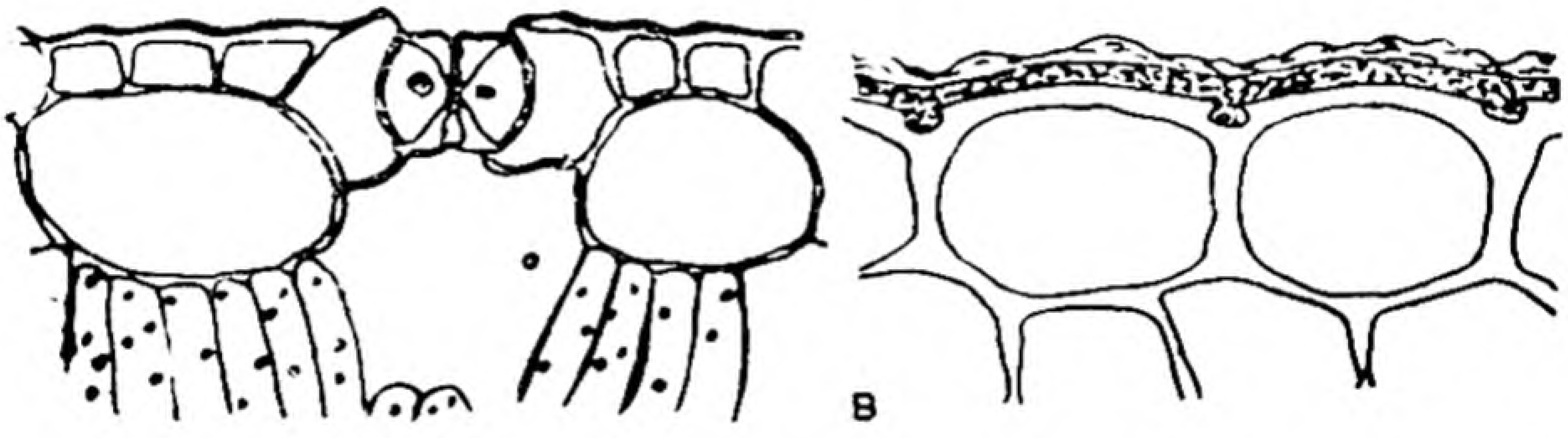


FIG. 2.20: Cuticle of the leaf of banana (Musa). **A**, section of upper, epidermis through stomatal region, showing extent of cuticle over guard cells; **B**, section through pulving band showing "pegs" of cuticle over anticlinal walls of epidermal cells, freedom of cutlcle between pegs, and waxy layer on surface of cuticle. (After Skutch.)

The surface of the cuticle is commonly smooth—always so in early stages—but when mature it may be roughened by cracks, by the sloughing of scales or minute particles from the surface, and by small nodules or slight ridges and irregularities (Fig. 2.19). Many so-called "cuticular pegs and ridges" are projections of the wall of the epidermal cells coated by the cuticle (Fig. 2.19). Such are the minute longitudinal flutings of the surface or stems and petioles of some herbs, for example, *Rumex*. The term "cuticular pegs" has been incorrectly applied also to the cutinized radial walls of eipdermal cells which, when seen in sections, appear as inward projections of the cuticle.

The floral parts, especiallay petals and stamen hairs, of some plants are reported to have in early stages a wrinkled cuticte, the wrinkles appearing as minute folds when the deposition of cutin is rapid and growth of the epidermis in area is slow. The folds are said to form a "reserve surface," and later, when growth of the organ is very rapid, are stretched out or reoriented. Over the mature epidermis the only folds remaining are those whose axes lie in the direction of greatest growth of the cells beneath. The radiating striations of the cuticle of papillate epidermal cells of petals are said to represent this condition.

The cuticle commonly adheres closely to the surface of the epidermis but has been reported to lie free and losssely over she walls in some floral harms and to be loose, but pegged down to the epidermis over pulvinar swellings. where the surface undergoes great changes in curvature, as in the leaf of banana *Musa*) Fig. 2.20. The separation of the cuticle from the epidermis by an air space is said to be responsible for the silvery appearance of some leaves.

The development of the cuticle, *cuticularization*, is accompanied, or followed in many plants by *cutinization* of the cells below. The outer walls of the epidermal cells first modified. Thesemay be increased greatly in thickness becoming many times as thick as the other walls, and as the cuticle itself (Fig. 2.19 F, G). They may be so heavily impregnated with cutin that they are separable only with great difficulty from the layer of pure cutin, the cuticle. The radial and inner walls may similarly be cutinized and even the walls of the subjacent cells. Where the entire walls of the epidernal cells become cutinszed, the cells may die, but frequently they remain alive, with normal pits and plasmodesmata. The term cuticle is unifortunately sometimes incorrectly applied in a functional sense to the entire outer "waterproofing" layer, the cuticle plus all cutinized cells. The layer which is stripped from leaves a "the cuticle" is usually the cuticle plus cutinized parts of underlying layers.

In some wood stems wheae the epidermis persists for more than one year—for example, species of *Acer*, *Cornus*, *Kerria*—

the original cuticle is cracked or ruptured and is repaired by secretion of new material from beneath, or a complete new layer it formed (Fig. 2.19H, I).

Many fruits, such as plum, tomato, persimmon, and some varieties of apple (Fig. 2.19B), have, when ripe, a thick cuticle. This reaches full thickness only as the fruit attains mature size. The heavy layer aids in the conservation of water in the fruit after the supply of water ceases. Some of the keeping qualities of fruits are due to the presence of a heavy cuticle. Varieties of apples with thick cuticles keep better than those with tinner cuticles. Fruits grown in sunshine have heavier cuticles than those grown in the shade; and apples grown in a "dry year" have heavier cuticles than those brone on the same tree in a "wet year." In the drying of prunes the cuticle is chemically or mechanically perforated to hasten the drying.

Minute particles or rods of wax on the surface of the cuticle are responsible for the glaucous character of stems and leaves and constitute the bloom of fruits. Wax may occur in large particles, as in the bayberry, *Myrica*, or may heavily coat the leaves, as in the wax palms.

3

Tissue and Tissue System

PLANT HISTOLOGY

The study of plant tissues and the cells which compose them is termed *plant histology*. One important point must be made at the outset, namely that the study of plant histology is more concerned with the architecture of the cell in relation to the function which is performs than with its internal composition, and therefore it is the study of cell walls rather than the study of the whole cell. Moreover, as we shall see later, many of the types of cells which form tissues are really dead structures, containing no protoplasm, although they still serve important functions. For this reason the amount of material which is actually living and growing in a higher plant is a varying and uncertain quantity, depending on the proportion of living to non-living cells. This proportion is greatest in early life and it diminishes progressively with age. In the embryonic stage all the body cells are living and actively growing, but in the course of development the growth activity becomes very soon localized, particularly at the tips of shoots and roots, where the tissues retain the embryonic character throughout the whole life of the individual. Plants thus present an opposite condition to that in animals, in which growth is general in all organs until a specifically limited size is attained, after which it ceases. In plants, on the contrary, growth is localized from an early stage, but is maintained at these points more or less continuously. The size reached by a plant is therefore, in favourable circumstances, simply a function of its age.

TISSUES AND TISSUE SYSTEMS

Among many lower plants there is little differentiation in kinds of cells; only simple vegetative and reproductive cells are present. Groups or masses of these cells that are alike in origin, structure, and function form *tissues*. The plant body consists of "vegetative tissue" and "reproductive tissue." In the higher plants the body is complex in cellular structure, made up of many kinds of cells, of most varied form and function and of different origin. For these plants a definition of "tissue" that is less rigid than one that requires similarity of origin, structure, and function is necessary. Morphologically, a *tissue* is a continuous, organized mass of cells, alike in origin and in principal function. Within a tissue there may be great diversity of cellular form and function, but the cells that make up a tissue must be contiguous (not dispersed among other cells) and must form a structural part of the plant.

Classification of Tissues

Tissues are classified on several bases—position in the plant body kind of constituent cells function, method or place of origin, stage of development. Each classification is made primarily or wholly on one of these bases. The classification can be divided into those of descriptive anatomy and morphology and those of physiological anatomy. Continuity of cells in the tissue is essential from the morphological viewpoint, whereas it is not important from the physiological viewpoint where function alone binds cells together as a tissue. This major difference in basis can be made clear by examples. Of the morphological tissue wood, or *xylem*, only part—the actual conducting cells—forms the "wood" of physiological anatomy, the supporting cells belong to a different tissue, the storage cells to still another. Morphologically the epidermis forms a single tissue, but in physiological treatments the majority of the cells of the epidermis are grouped with the periderm to form "dermal tissues," and the guard and accessory cells of the stomata form a part of the "aerating tissues." Scattered and isolated cells and groups of cells form many of the tissue of physiological anatomy.

Tissue Types Based on Stage of Development

Meristematic and Permanent Tissues

Tissues are distinguished as "meristematic" and "permanent" on the same basis as are cells. Meristematic tissues are immature tissues in which growth is taking place; permanent tissues are those in which growth has ceased, at least comporarily. permanent tissues—as a whole or in part—may again become meristematic.

Tissue Types Based on Kind of Constituent Cells

Simple and Complex Tissues

From the standpoint of number of kinds of cells making up a tissue, tissues are *simple* or *complex*: they are simple when homogenous, consisting of one kind of cell; complex when heterogeneous, consisting of more than one kind of cell. This classification aids in detailed descriptions of tissues. There are very few simple tissues in plants. The common ones are *parenchyma collenchyma* and *clerenchyma*. These terms are applied also to cells; for example, a cell may be called "a parenchyma cell." Such a cell is either a unit of the imple tissue, parenchyma, or a cell with the characters of the cells of parenchyma but a member of a complex tissue. Parenchyma and clerenchyma cells are common constituents of complex tissues; collenchyma cells do not occur with other kinds of cells. She adjectives "parenchymatous" and "sclerenchymatous" indicate cells which possess come of the characters of the cells of parenchyma and of sclerenchyma, rspectively, but which do not belong definitely in those tissues. There are parenchymatous fibres, sclerenchymatous cork cells, etc.

The conducting tissues, *xylem* or *phloem*, are the prominent complex tissue types. They need separate discussion here because of their great complexity and variety and because of their structural and functional convinience. Other complex tissues can be interpreted as combinations in parenchyma and sclerenchyma or modifications of these tissues. Xylem and phloem are considered in this chapter as to general structure and function; they are treated further in other chapters.

Parenchyma

Simple vegetative tissue, that is, tissue which is usually not complex or elaborate in structure or form, like that which takes up the body mass of lower plants and the nonspecialized portions the more complex plants, is called *parenchyma.* Parenchyma is a rather, closely used term in that it is applied to all generally unspecialized and firly simple tissues which are concerned largely with the ordinary vagetative activities of a plant. Parenchyma is, obviously, phylogenetially the primitive tissue since the lower plants have undoubtedly given are to the higher plants through specialization and since the single type in the few types of cells found in the lower plants have become by specialiation the many and elaborate types of the higher plants. Further, all cristematic tissue is unspecialized. Hence, it is parenchyma-like—in face is often called parenchyma—and it can be said that, ontogenetically also, parenchyma is the primitive tissue.

The general characters of parenchyma cells are diameters essentially equal, walls thin, protoplast present, and a capability for cell division even when the cells are permanent cells (Fig. 3.1). Exceptions occur in all these characters. Parenchyma makes up large parts of various organs in many plants. Pith, the mesophyll of leaves, and the pulp of fruits consist chiefly of parenchyma; the cortex and pericycle are often wholly or in large part parenchyma and parenchyma cells occur freely in xylem and phloem.

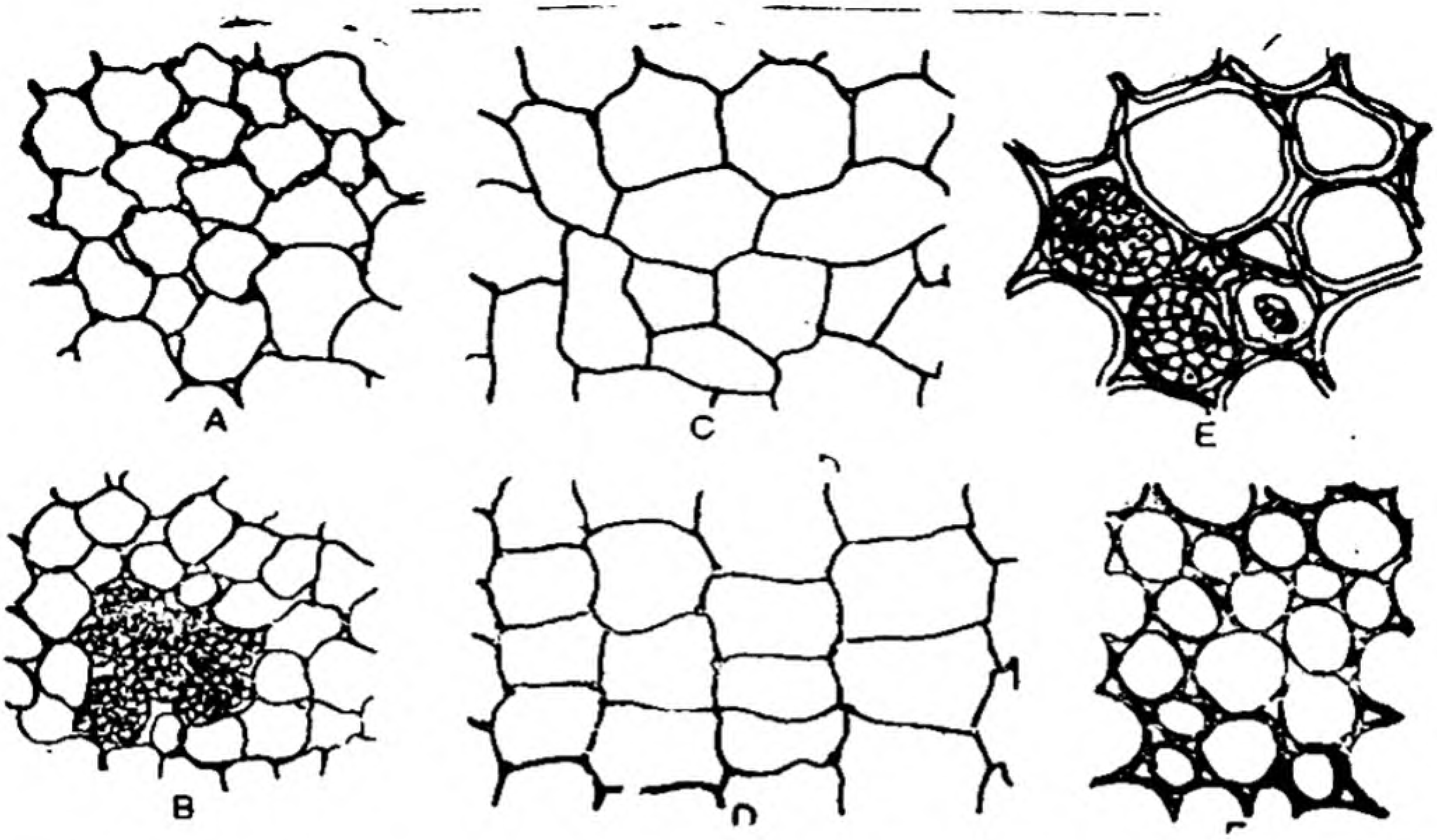


FIG. 3.1: Parenchyma. A, from pith of rhizome of Polypodium vulgare; B, from cortex of root of Asclepias incarnata, the cells filled with starch grains; C.D. from pith of Zea, transverse and longitudinal respectively; E, thick-walled, ligkified cells from pith of twag of Castinea dentata, the cellf containing starch grains or crystals; F, thick-walled cells from pith of Clematis virginiana.

In early studies of plant structure all tissues were divided on the basis of general from and function into *parenchyma* and *prosenchyma.* The latter was distinguished from parenchyma chiefly by its edongate, pointed, thick-walled cells and its specialized fuactions of support, protection, and conduction. But entirely different types of tissue are present in "prosenchyma" and the term became obsolete. For conveninence, cells and tissues are still frequently described as "prosenchymatous" in contrast with "parenchymatous."

Collenchyma

Collenchyma is a living tissue and is composed or more of less elongated cells with thick primary *non lignfied walls.* Just as in parenchyma, even in collenchyma the protoplast as is living. The word collenchyma is derived from the Greek word *colla,* meaning glue, which refers to the thick glistening wall of collenchyma.

Origin

According to Ambronn (1881), Haberlandt (1914) and Majumdar (1941), collenchyma originates jointly with the vascular tissues from the procambium. According to Esau (1936) however, collenchyma independently originates from the procambium.

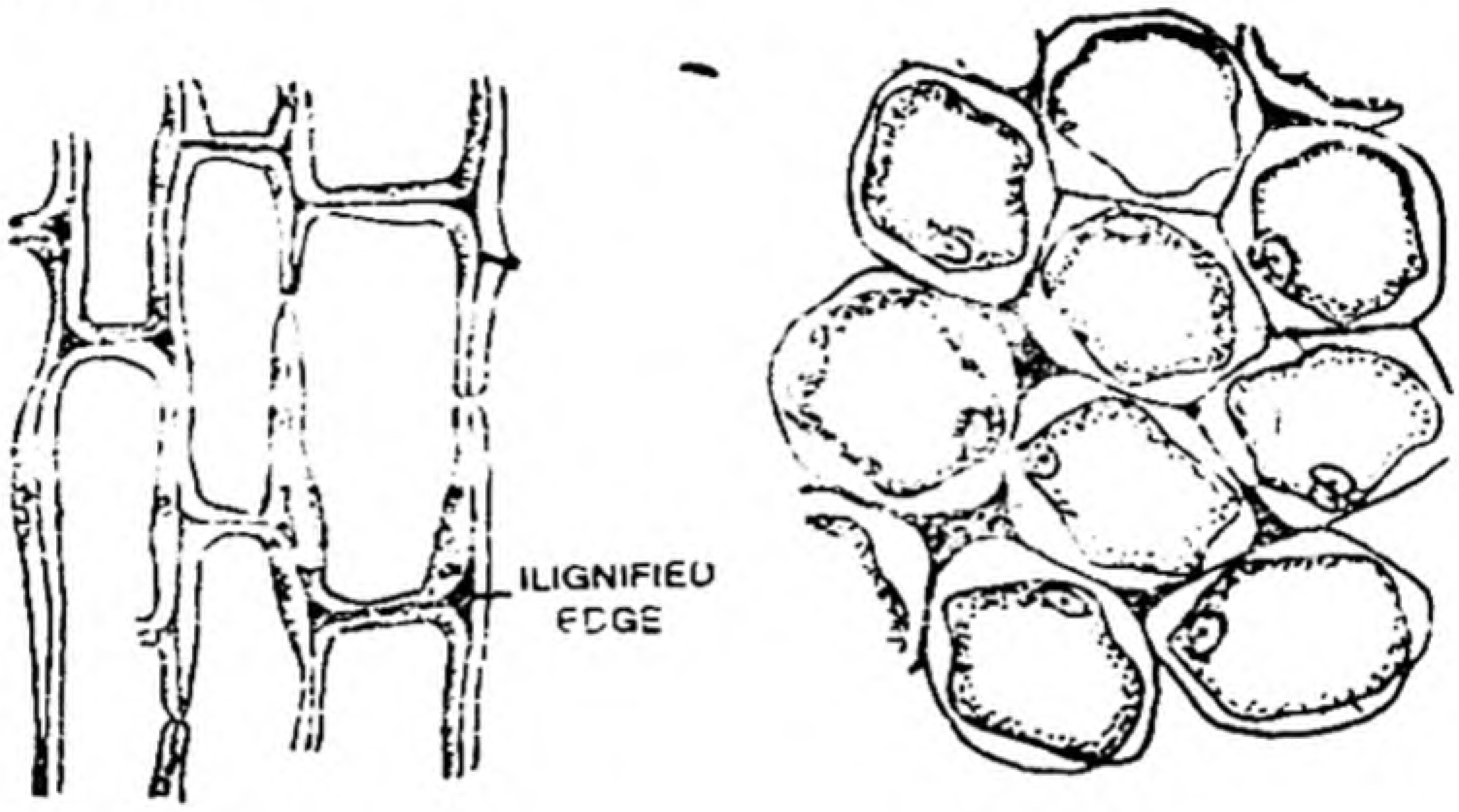


FIG. 3.2: Permanent tissues, Collenchyma. A-LS., B-T.S.

Distribution: Collenchyma characteristically occurs in the peripheral position of stems, leaves, floral parts etc. It may also occur in root contex particularly if it is exposed to light. In the stems and leaves of dicotyledons, collenchyma is hypodermal, situated immediately below the epidermis or separated from it by one or two layers of parenchyma. Collenchyma is absent in the stems and leaves of many monocotyledons.

Cells Shapes: Collencyma cells are considerably elongated. Sometimes, cells are 2 mm., long resembling fibres. The shorter collenchyma cells are prismatic and appear polygonal in a transection (Fig. 3.2). In a long section the cells appear rectangular.

Cell structure and function. The structure of the cell wall is the most characteristic feature of the collenchyma cells. The thickenings are deposited unevenly, mainly deposited at the corners where several cells join together. In long sections collenchyma cells show thin and thick wall portions depending on the direction of the cut. The walls contain mainly of cellulosic and pectic compounds and consist much water. Ultrastructurally the thickenings of collenchyma show an alternation of layers of longitudinally oriented microfibrils and non-cellulosic material.

Collenchyma cells have living protoplasts and may have chloroplasts. Chloraplasts are less in number in long and narrow cells and more in cells which approach parenchyma in form. Tannins may be present in collenchyma cells

Collenchyma is primarily a mechanical tissue adapted for support of growing organs. The thick walls and close packing, make collenchyma a strong tissue. At the same time the peculiarities of growth and the structure of its walls impart a considerable tensile strength with flexibility and plasticity.

In many stems, support in early stages is given in large part by a soft but strong tissue known as *collenchyma*. Important characteristics of this tissue are its early development and its adaptability to elongate in the rapidly growing organs, especially these of increase in length. When it becomes functional, no other strongly supporting tissues have appeared. It consists of elongate cells, various in shape, with unevenly thickened walls, rectangular, oblique, or tapering ends, and persistent protoplasts. The cells overlap and interlock in varying degrees, forming strands similar to those of fibres. The walls consist of cellulose and pectin and have a high water content. (The presence of pectin probably accounts for the high water-absorbing capacity). They are plastic, extensible, and readily adapted to rapid growth. The strands are at first of small diameter but are added to as growth continues, from surrounding meristematic tissue. Cells on the borders of the strands may be transitional in structure, passing into the parenchyma type.

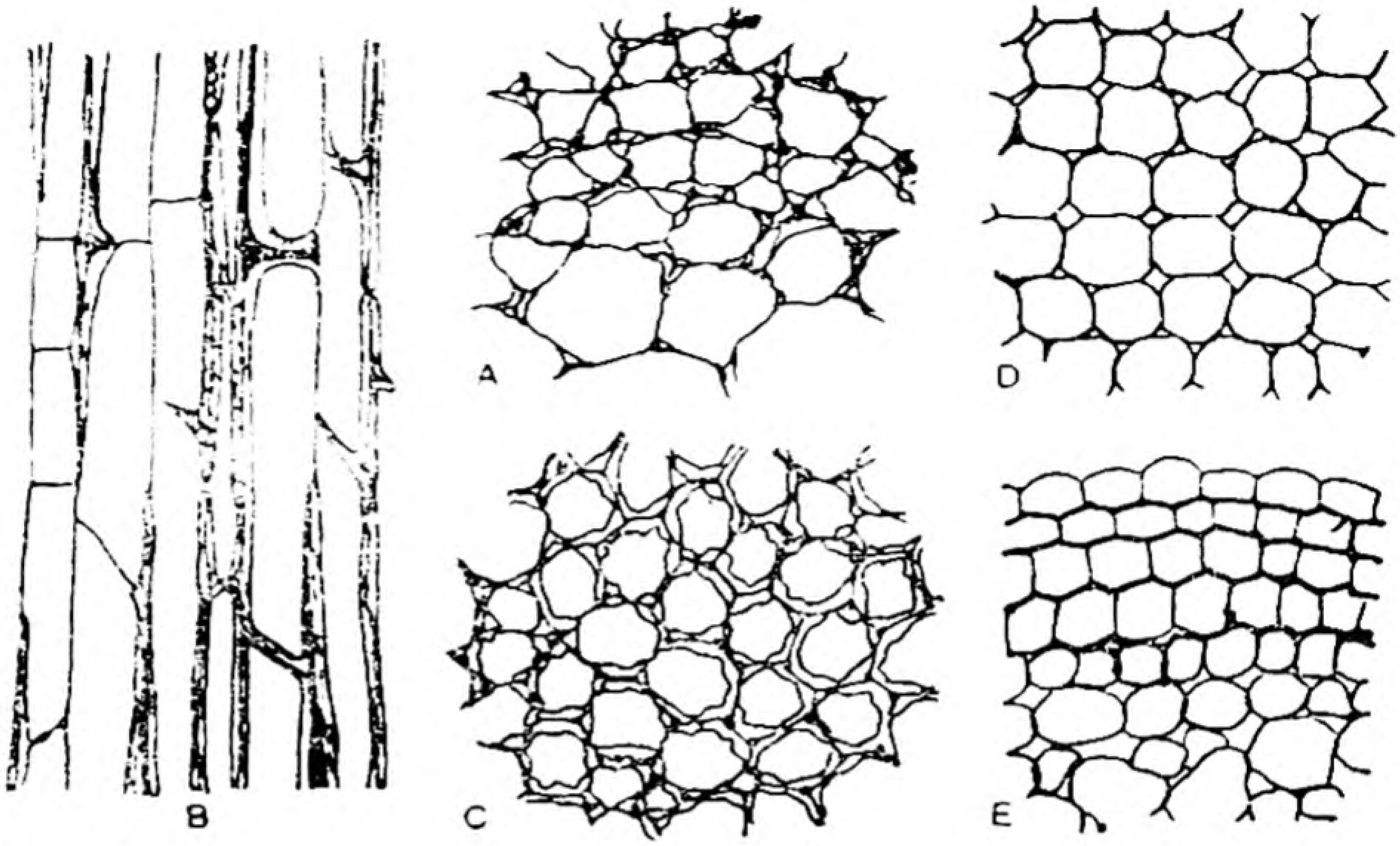


FIG. 3.3: Collenchyma, A.B. transverse and longitudinal sections from stem of Sclanum suberosum; C, D, E, transverse sections from stem of Abutilon Theophrasti, stem of Asclepias syriaca and petiole of Asarum canadense, respectively.

The areas of greater wall thickness are in the form of longitudinal strips Fig. 3.3B which occupy the corners of the cells (Fig. 3.3 A.C.) cover the tangential walls (Fig. 3.3 E) or are confined to those parts of the walls that about on intercellular spaces (Fig. 3.3 D). Where spaces are surrounded by thickened wall strips, hollow rod-like structures are formed, and the collenchyma appears to consist of thick-walled calls among thin-walled cells (Fig. 3.3 D).

Sclerenchyma

Another type of supporting tissue, which is also in large measure protecting, is *sclerenchyma*. The cells of this tissue, in contrast with those of collenchyma have hard, usually lignified walls with a low percentage of water. At maturity they usually have no protoplasts. The walls are uniformly and strongly thickened. In shape and size, scelerenchyma cells are most diverse, but two general types are recognized—*fibres* and

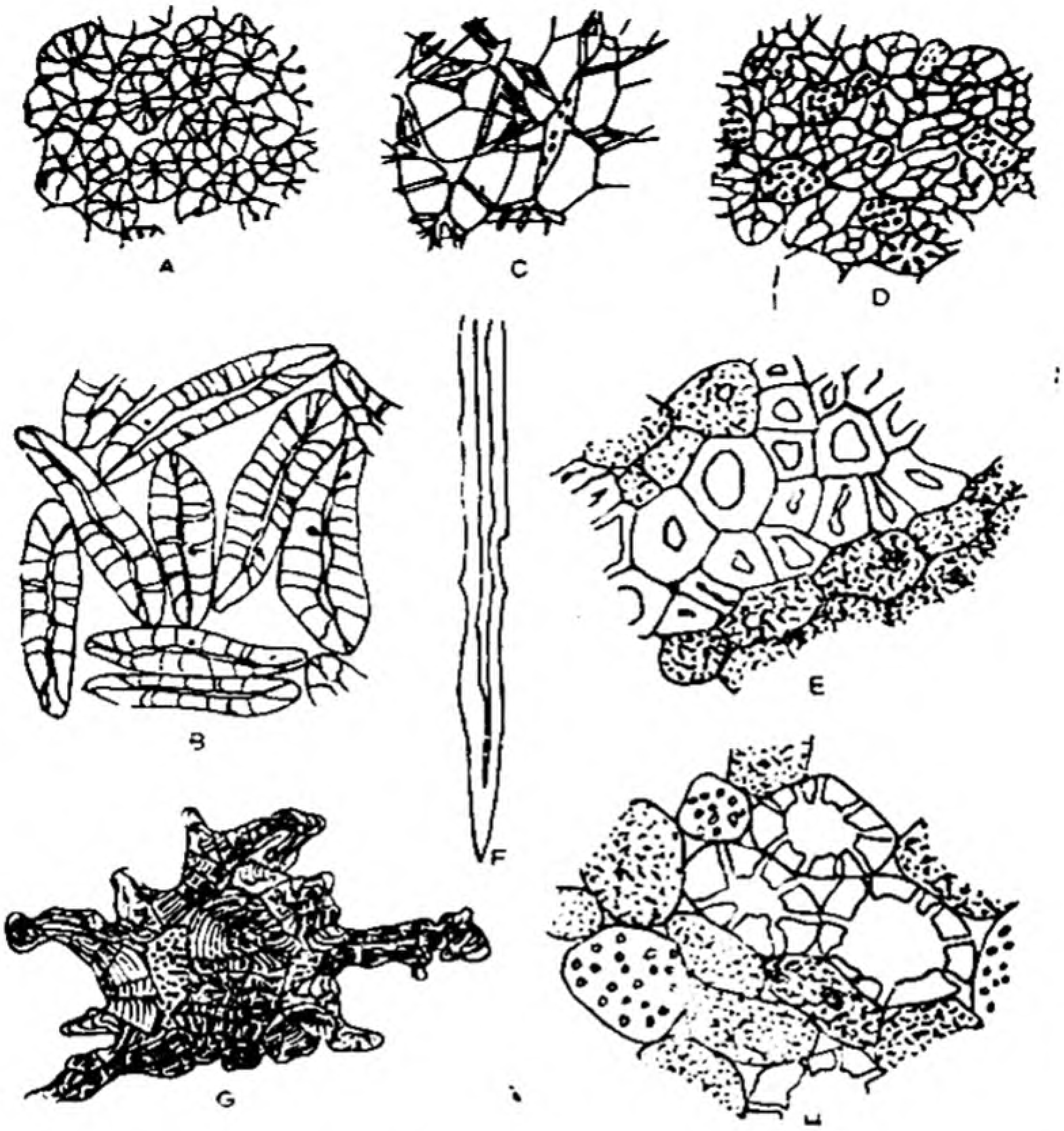


FIG. 3.4: Sclerenchyma, A, B, transverse and longitudinal sections of elongate sclereids from endocarp of Cocus nucifera (coconut shell); C, Sclereids from endocrap of Crataesus showing the pits fused in groups; D, sclereids ("grit cells") from the fleshy pericarp of Pyrus communis; E. fibres of the primary pholem region of Cannabis in transverse section; F, longitudinal section of part of one fisher shown in E; G, irregular sclereids from the phloem of Tsuga; H. sclereids and parenchyma cells from the stem cortex of Dracaena fragrans.

sclereids. The distinction of these forms of sclerenchyma is one of convenience in description; it has no morphological significance. Intermediate forms are many (Fig. 3.4B), and both types may occur in the same tissue, even intermingled, and serve the same general function.

Fibers. Fibres are elongate sclerenchyma cells, usually with pointed ends. Chemically, the walls are usually lingified, although there are fibres with walls largely of cellulose and others with gelatinous walls. The pits of fibres are always small, round or slit-like in outline and, in mature cells, unless a protoplast is present, are doubtless functionless. Pits may be numerous but are common rather few in number, and in fibre types with excessively thick walls they may be few or present only as vestigial structures. The lumen of fibres is small; it often is a more channel through the center of the fiber, and this opening may be blocked in spots so that at certain levels in the fibre no lumen exists, only a line or spot representing, in cross section, its former position. In the development of fibers the protoplast often becomes multinucleate. In most kinds of fibres, however, the protoplast disappears as the cell reaches maturity, and the permanent cell is dead and empty. Fibres that retain their protoplasts and other types of fibres are discussed in more detail under the tissues in which they occur.

Classification of fibres. If the usual and loose use of the term "fibre" such as is covered by the above definition—is accepted, fibres may occur in nearly all parts of the plant. They are most generally found and are most abundant in the cortex, pericycle, phloem, and xylem. Morphologically, there are two distinct types. The fibres of the cortex, pericycle, and phloem possess simple pits and are thus different from those of the xylem which have bordered pits (although these pits may be so reduced as to be essentially simple) since xylem fibres are morphologically reduced tracheids. Fibres are sometimes divided into classes: "bast fibres" and wood fibres. These groups are essentially the same as the two just discussed, but the terms are poor since the word "bast" is involved and has, unfortunately, several uses. In its most common use, the term "bast" is synonymous with phloem or refers to fibres of the secondary phloem. "Bast fibres" in the above classification include fibres of the cortex and pericycle as well as of the phloem. Fibres can best be designated by means of the tissue or region in which they occur, as cortical fibres, pericyclic fibres, phloem fibres, wood fibers, etc.

Fibres occur singly or in small groups scattered among other cells. Usually they form strands or sheets of tissue extending longitudinally for considerable distances. Their value as strengthening tissue is largely due to their arrangement in these long masses and to the overlapping and interlocking of the cells. They serve also to give general firmness to tissues.

Fibres develop in two ways, In those only a few millimeters long—for example, those of the Manila hemp, *Agave*, *Sansevieria*—all parts of the cell are always at the same stage of development at the same time. As in most cells, the secondary wall is deposited simultaneously throughout the cell when full size is attained. In much longer fibres, for example, those of flax and hemp, the cell elongates apically, keeping pace with the growth of surrounding cells, and the secondary wall develops in part of the cell while the apex is soil growing.

The term "fibre" is popularly applied to various plant structures that are not morphologtcally fibres: hairs (cotton); strands of cortical or phloem fibres (flax, hemp): foliar vasetar bundless with their sclerenchyma sheaths or caps, or the caps alone (Manila hemp, New Zealand flax, sisal); strands of collenchyma (celery); wood cells generally (paper pulp); fragments of leaves and woody tissues (various drugs); bits of "seed" coats (wheat flour).

Sclerids. These are short isodiametric cells (Fig. 3.5 A and B) but sometimes may be elongated also. The cell walls are very thick and lignified but the cells do not have tapering ends. The cells are dead and have a very narrow lumen. The secondary walls show prominent pits which are simple. Sometimes there is light overarching of the secondary wall over the pit chamber. The lignin deposition is uniform, but may be uneven as in the seed coats of some *Leguminosae*.

Sclerids arise through a belated sclerosis of ordinary parenchyma cells or directly from cells called sclerid primordia.

Stone cells are also called *brachysclereids* (Fig, 3.5 A, B).

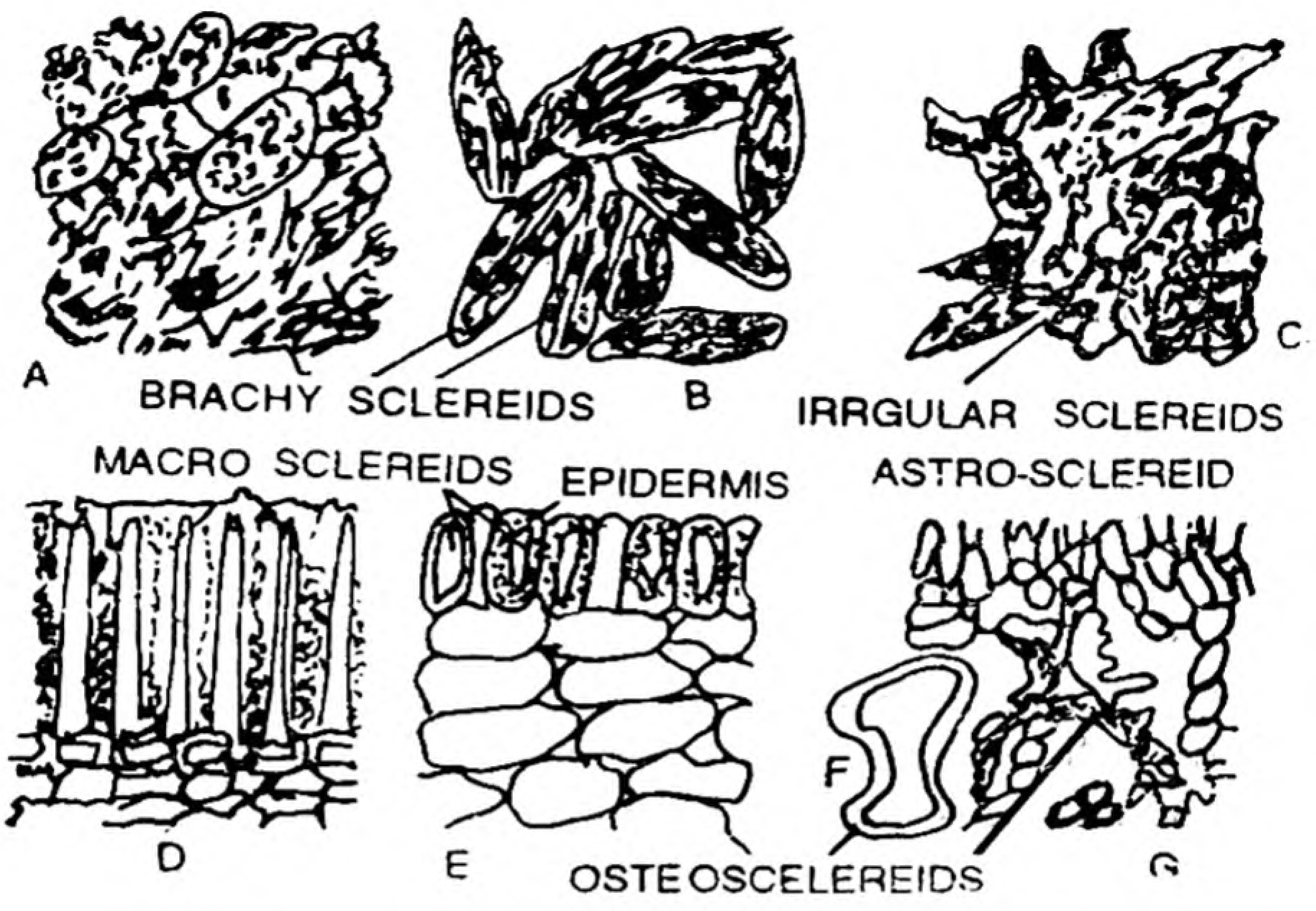


FIG. 3.5: Permmanent tissues., Type of Sclereids, A and B. Brachy. sclereids, C. Irregular sclereids D and E. Macrosclereids, F Osleosclereid, G. Astrosclereid.

Sclereids more extreme in form have been distinguished as: *macrosclereids* (rod cells) Fig. 3.5 D, E) more or less columnar in shape, constituting the "palisade layer" of many seeds and fruits and occurring in some xerophytic leaves and stem cortices; *osteosclereids* (bone cells) of brone-like or barrel shape (Fig. 3.5 F constituting hypodermal layers in many seeds and fruits and frequent in xerophytic leaves; *astrosclereids* (stellate cells Fig. 3.5 G with extreme lobes or arms, in leaves and stems of xerophytic organs; *trichosclereids* ("internal hairs"), branched sclereids with lobes projecting like hairs, into intercellular spaces, in leaves and stems of hydrophytes. The last two types are structurally closely alike. A classification of sclereids on shape and structure is of little value. The character of "branching" or "non-branching pit cavities," which has been used, in part, in distinguishing sclereid types, is largely dependent upon the thickness of the cell wall. Sclereids may occur almost anywhere in the plant body but are most abundant in the cortex, in the phloem, and in fruits and seeds. Like fibres, they occur singly, in groups of a few cells, or in large masses. The hard parts of seeds, nuts, and hard fruits generally are made up largely of stone cells of various types. Where stone cells are scattered, they merely give firmness, as in leaves and the flesh of fruits. In masses, they give hardness and mechanical protection, as in many kinds of bark and in the shells of nuts.

The wall of sclereids is very thick and strongly lignified. Occasionally, it is suberized or cutinized. The pits are very small with round apertures, and their cavities have the form of more or less branching canels because of the fact that, as the area of the cell wall is reduced on the inside by the increased thickening, the pits are brought together (Fig. 3.4 A, C). Two or oven several pits thus fuse to form one structure which has only one aperture in each cell but has as many arms as there were original pits. Sclereids are usually dead cells. The shriveled remains of protoplasm and inclusions of the protoplast, such as tannin and mucilage, may be present.

THE IMPORTANT COMPLEX TISSUES

The structural and functional prominence of the vascular system renders its tissues of great importance as tissue types. These vascular tissues are, in fact, the only complex tissues which need separate discussion; all others may be interpreted as combinations of parenchyma and sclerenchyma and, of modifications of these tissues. The following treatment of vascular tissues is of general nature only since a description of xylem and phloem as primary and secondary tissues and as specially modified in various organs.

Xylem

The Tracheid

The fundamental cell type in xylem is the *tracheid*. The tracheid is an elongate cell with tapering ends (Fig. 3.6) which when matures is nonliving, that is, without a protoplast. The walls are hard but not thick and are usually lignified. In cross section the

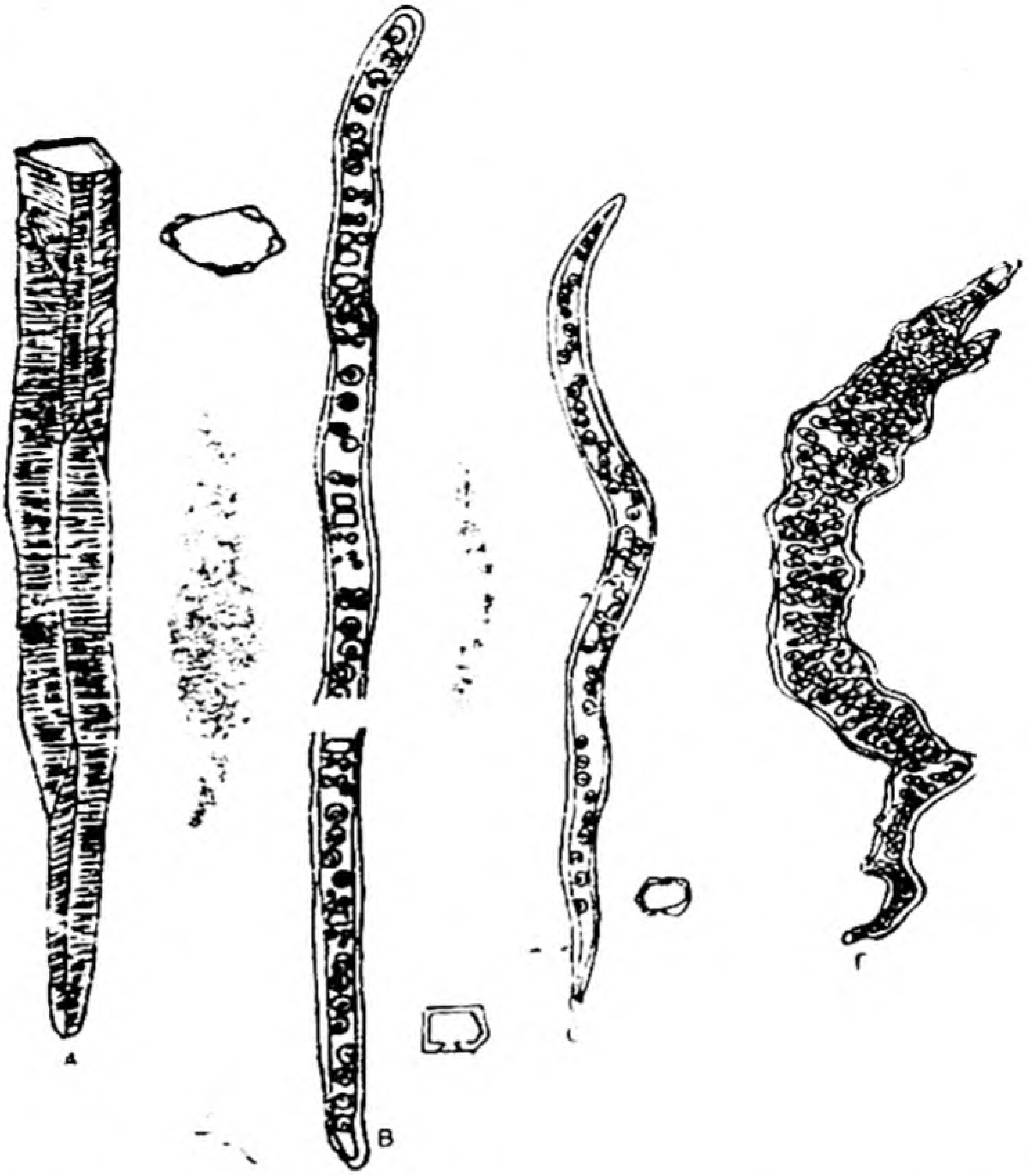


FIG. 3.6: Tracheids. A, from Woodwardia virginica, one-sixth of cell shown; B, from Pinus Strobus, one-third of cell shown; C, D, from Quarcus alba, C, a normal, and D, a flattened and distorted tracheid from the spring wood. (The illustrations of tracheids are drawn to scale and to the same scale as are those of fibres and vessels.)

tracheid is typically angular, though more or less rounded forms occur. The tracheids of secondary xylem, owing to their method of arrangement, have fewer sides than those of primary xylem and are often more sharply angular. The ends of the tracheids do not taper uniformly to the tip in all planes, but the tapering is confined largely to the radial plane and usually to one side of the cell only. The end of a tracheid of the secondary wood is more or less chisel-like. The tapering is seen in tangential sections of the tracheid; radial sections do not show tapering, the end of the cell in such sections being rectangular or somewhat rounded. The pits are abundant and of the bordered type, though they vary in size, in outline, and in distribution over the walls. The lumen of a tracheid in large and free of contents of any kind. The tracheid is apparently well adapted structurally to its functions which are water conduction, primarily, and support, secondarily. It is a long, empty, firm-walled tube extending parallel with the long axis of the organ. It is in communication with contiguous tracheids—as well as with other types of cells, living and nonliving—by means of numerous, well-developed pits. These thin areas permit ready diffusion into adjacent cells. The arrangement of tracheids is always such that contiguous cells overlap at least over the tapering portions, and over these areas of the wall, the pits are often abundant. Channels for longitudinal canduction are thus provided through a series of lumina which form a more or less direct line or an anastomosing system.

The continuity of the walls of the tracheid. The individual lumina are shut off from one another by thick walls and, in the pits, by the closing membranes. The closing membrances. however, except in the central region, the torus, are very delicate and, at least in some of the conifers, are perforated. In *Larix* and *Sequoai*, for example, the perforations, though microscopically minute, are so numerous that the torus is suspended by a meshwork of strands. The opening in perforated pit membranes are not readily seen unless the membrane is well stained, but their presence is demonstrable by the passing of solid particles such as those of carbon in India ink under pressure from one tracheid to another through the pits.

Pits of Tracheid Walls. The position of pits in the wall of the tracheid and the size and shape of pits depend upon the position and nature of the contiguous cells. The various larger plant groups have more or less constant types of bordered pits which have characteristic shape, torus, and extent of border. The ferns and the clubmosses have transversely elongated pits with narrow borders and little or no torus. The pits lie close together, covering the wall, giving it a ladder-like appearance (Fig. 3.6 A), and the cells are called *scalariform tracheids*, or, better, *scalariformpitted tracheids*. The former term is undesirable in this connection since it is also used with other meanings and where definite pits do not occur (protoxylem). In other gymnosperms and most angiosperms the bordered pits are chiefly rounded with wide borders (Fig. 3.6, B, C); those of the angiosperms are mostly much the smaller. The best development of the torus is in the gymnosperms where the bordered pit probably reaches its highest development. The closing membrane of these pits is so constructed that its position in the pit cavity can be readily changed from a median position to a lateral one with the torus closely appressed to the aperture of the pit. A valve-like action is thus secured, the pit being freely open when the torus is in the median position, diffusion—or in perforated closing membranes, direct passage—taking place around the torus through the peripheral part of the closing membrane. When one of the pit moths is closed by the placing of the torus against it, direct passage is largely or wholly shut off, and diffusion must take place through the thicker and denser torus. Thus, apparently by changes in the position of the closing membranes of bordered pits, there may be some control over the passage of fluids in xylem. It is significant that the type of pit in which this control of the passage of fluids is present is characteristic of water-conducting cells and does not occur elsewhere; also that in the structurally reduced pits of fibre-tracheids and fibers the pit membranes have lost the capability for movement. In simple pits no structural complexity occurs.

Function of the Tracheid. The tracheid is structurally adapted, both in lumen and in wall, to the function of conduction. The thick and firm walls of tracheids also aid in support, and, where there are no fibres or other supporting cells, the tracheids play a prominent part in the support of an organ. For this the overlapping and interlocking of the cells and their union into strands and cylinders are as important as are thick walls.

Tracheids alone perhaps made up the xylem of very ancient plants, but in living forms wood is a complex tissue. with parenchymatous wood rays even in the simplest forms of secondary xylem; and primary xylem (as a morphological tissue unit) alwuys contains parenchyma cells. The more complex types of xylem may contain several kinds of cells—tracheids, *fibres* of one or more kinds, *ressels* of one or two types, parenchyma cells (known as *xylem parenchyma* or *wood parenchyma*), and *wood-ray parenchyma*.

The trachied clearly serves both as a conducting and as a supporting cell. But evolutionary development in xylem has resulted in a specialization of this once simple tissue in such a way that the original functions of the tissue have become segregated in distinct cell types—support to fibres of various kinds and conduction (of an apparently more efficient type) to vessels. The new function of food storage has been acquired, and this is carried on by wood parenchyma. The wood-ray parenchyma is also concerned with food storage; the ray is, however, probably primarily concerned with laterial conduction. In complex xylem in which the definitely dissociated functions of support and conduction are given over to fibres and vessels respectively, trachieds are usually not found. But in the wood of *Quercus* and some other general all three kinds of cells are present.

The Term "Vessel"

Since the perforations of the cell occur usually in the end walls, the development of end walls trasverse to the long axis of the cell brings a series of cells into a definite tube-like system, which provides for transmission in a more or less nearly straight line. Such a condition is in contrast with the indirect lines of conduction in a group of tracheids. A tube-like series of cells thus formed has long been known as a *vessel*, or *trachea*.

Both these terms have unfortuately been applied in two ways: to the series or system of cells, and to individual cells.

with perforate walls that serve in direct water conduction. The former has the support of priority and long usage and should be continued. The unit cells making up such a series are called *vessel elements*, *vessel members*, or *vessel units*. (The term "vessel segment," which has been in frequent use is inappropriate and should be discarded because it implies that the series is a unit which has been divided to form the cells.) Some difficulties arise in the application of these terms under all conditions. In plants in which the water-conducting cells are tracheid-like in shape, union of the cells forms a mesh-system, and vessels of the tube type are not present. The terms "vessel element" and "vessel member" can be applied here to the individual cells. Vessels are present in the sense of continuous series of fused, perforate cells although individual tubes cannot be distinguished.

Types of Vessel Perforations. The openings in vessel-element walls, known as *perforations*, are restricted to the end walls except in certain slender, tapering types where definite end walls cannot be distinguished, and they are said to be present on the side walls (Fig. 3.7 A, E). Most vessel elements are perforated in two areas, one at each end, but three and even four such areas occur in some vessel elements. Where there are three or four, connections are made with other vessels or with branches of a meshwork system of vessel elements. The more or less definitely delimited or outlined area of the wall in which perforation occurs is the *porforation plate*. Commonly this is an end wall. The portion of the plate remaining after perforation has occurred is the *perforation rim*; the strips of cell wall between scalariform perforations are the *perforation bars*.

Perforation plates are described as having a *simple perforation* if there is but one opening; *multiple perforations* if there are two or more openings. Multiple perforations are grouped as *scalariform*, where the openings are more or less elongate and parallel; and *reticulate*, where the arrangement of the openings makes a mesh-like structure. The common

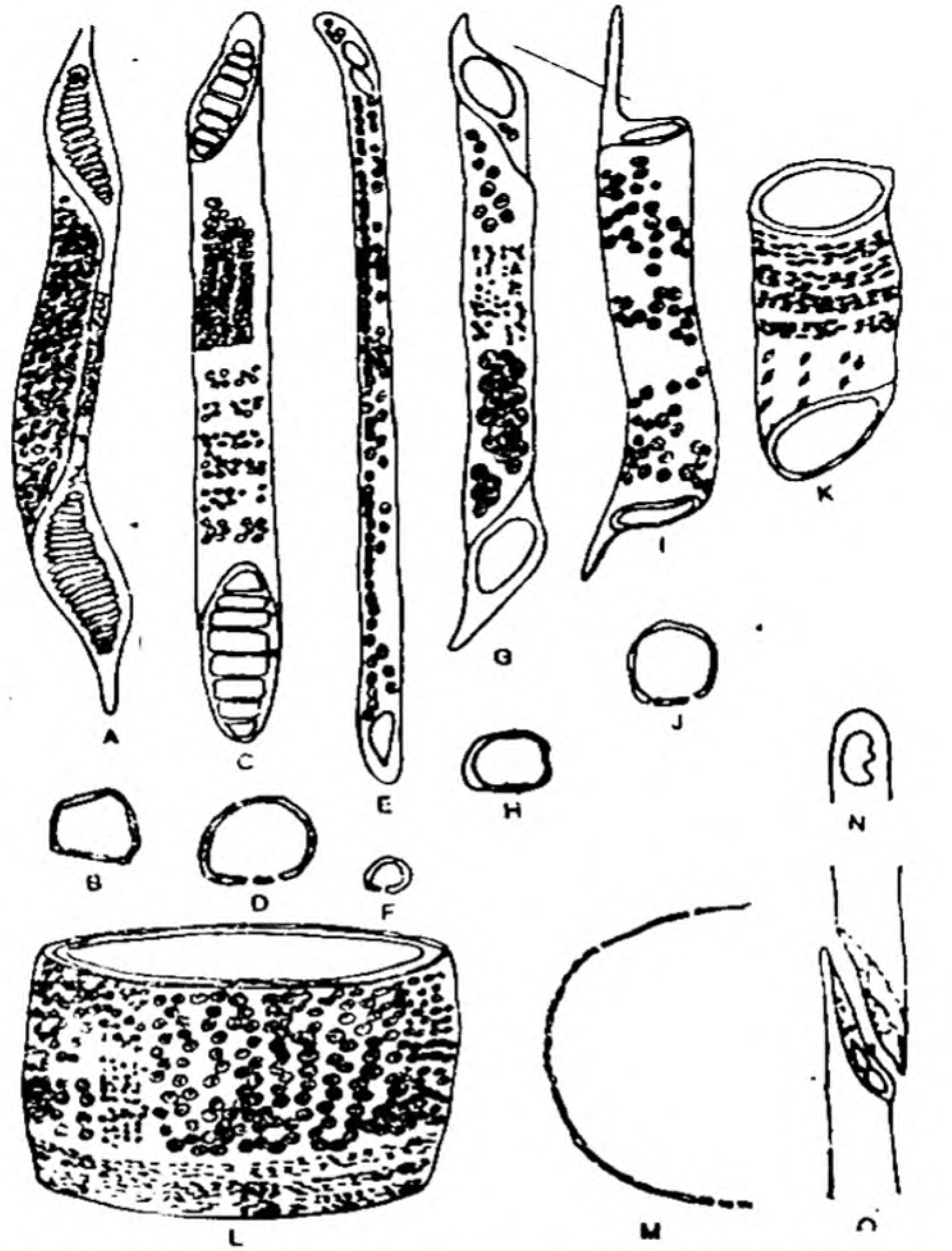


FIG. 3.7: Vessel elements in side view and cross section. A, B, from Betula alba; C, D, from Liriodendron; E, F, from Lobelia cardiualis; G, H, from Quercus alba; I, J, from Malus pumila; K, from Acer Negundo; L, M, from Quercus alba; N, end of vessel element from Lobelia showing perforation indicating derivation of porous vessel from scalariform; O, ends of vessel elements from Lobelia, showing method of union of elements in a series.

types are the simple and scalariform. The reticulate type is infrequent and not clearly separable from the scalariform. Small vessel elements occasionally have one type of perforation at one end, another at the other. Simple perforations are usually round but range in form in slender elements to narrowly elliptical. In annular and spiral vessels the perforation is of either type, but most frequently is simple. Commonly, end walls that are transverse have simple perforations, and those that are oblique have scalariform openings, but there are many exceptions. Phylogenetically the scalariform type is primitive and forms transitional to the simple are frequent.

Vessels are characteristic of the angiosperms; in only a few are they lacking—the Winteraceae, Trochodendraceae, and Tetracentraceae (which are primitively vesselless families) and in some xerophytic, parasitic, and aquatic genera where they have been lost in reduction. In many monocotyledons they are absent from the stemps and leaves; in others from either the stems or the leaves. They are present in some species of *Selaginella*; among the ferns, in two species of *Pteridium*; among the gymnosperms, in the Gnetales. In each of these groups the vessel has clearly arisen independently, in the angiosperms probably more than once.

Vessel Length and Width—Vessels extend for distances that vary with the kind of plant, the type of xylem, the type of vessel element, the location in the organ, and apparently with the rate of growth of the organ. The limits of an individual vessel are difficult to determine. In climbing plants and in trees with ring-porous wood and simple-perforate elements they may be several, perhaps many meters long. But they are often less than one meter and frequently only a few centimeters long. In a single tree they are apparently progressively longer from near the pith outward. It is highly doubtful that they extend "from root tip to stem tip" in any plant. Where branching and interlocking of elements occur, no question of length arises. Even the usually direct-series type may branch, and determination of unit length becomes uncertain. The terminal elements have but one perforation and taper at the blind end.

Although vessels characteristically are wide, the more slender forms may be even narrower than are typical tracheids. From this diameter they range to a maximum of somewhat over one millimeter. The wider vessels are characteristic of certain herbs such as *Zea*, many woody vines and lianas, and of some trees, such as, *Castanea*, *Quercus*, and *Fraxinus*.

Ontogeny of the Vossel. Vessels are formed from series of xylem mother cells—procambium cells or cambium derivatives—by the fusion of the cells end to end during the last stages of development. This fusion involves the loss of the end walls, or parts of those walls, so that the lumina of the series of cells are freely open into one another, and, with the walls, form a long tube (Figs. 3.8).

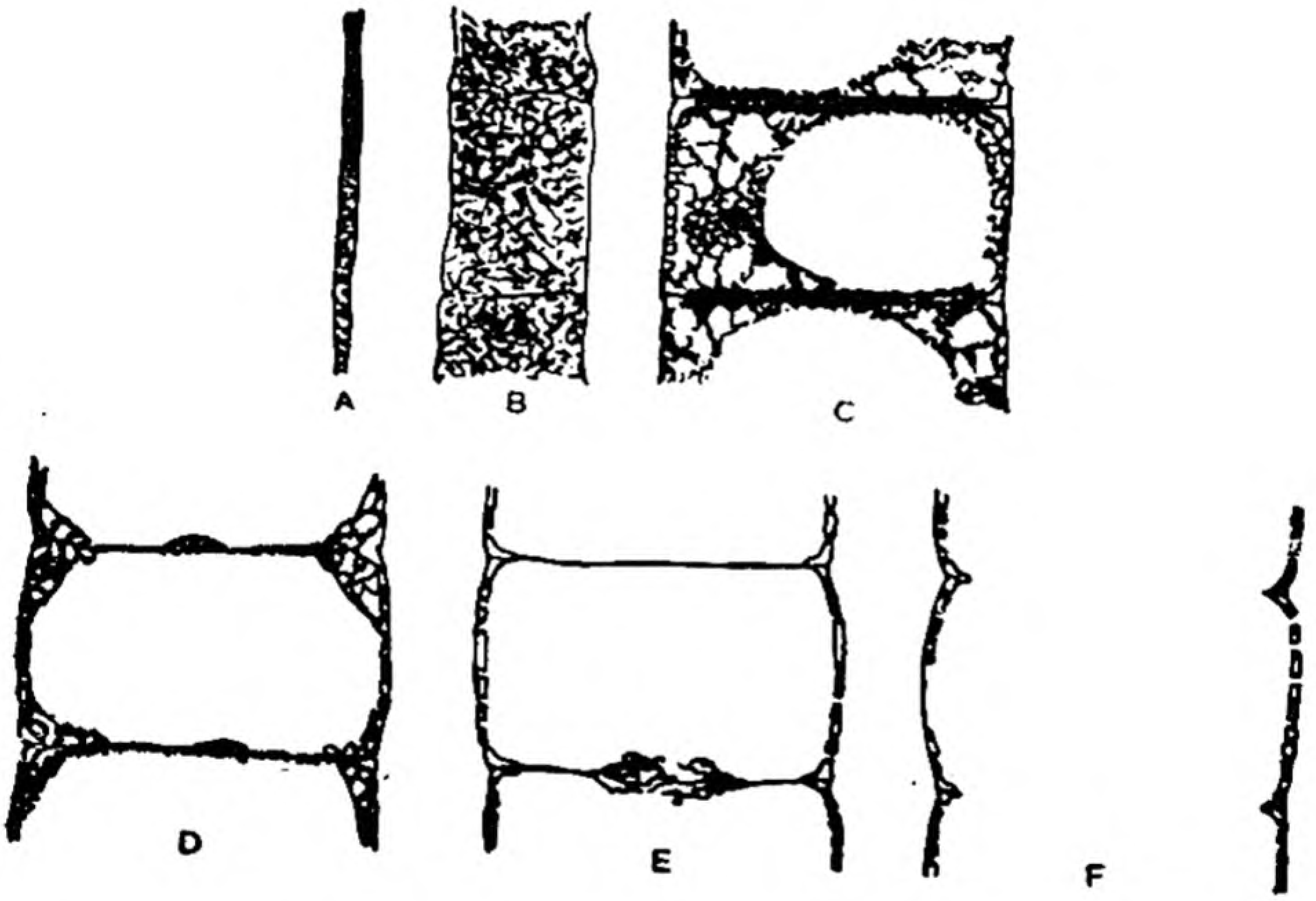


FIG. 3.8: The ontogeny of the vessel element in Robinia Pseudo-Acacia. A, the cambium initial; B; the cell much enlarged; C, the cell still further enlarged, the secondary wall well developed, except on the perforation areas where the primary is thickened, and the pits present; D, the cytoplasm restricted to the periphery, the nucleus adjacent to the wall where dissolution is occurring, the secondary wall removed from the pore areas; E, the cytoplasm lost, the very thin end walls distinegrating; F, the mature, perforated, empty cell.

From the meristematic stage the vessel elements enlarge rapidly, increasing greatly in diameter. Those with scalariform perforations and the more elongate, simply perforate types may increase in length somewhat, the tips forming 'talls' which penetrate between surrounding cells. Those that become of great diameter, and especially those developing from stratified cambium initials, do not elongate and may even become somewhat shorter.

During the rapid growth in cell size, the primary cell wall, although greatly and rapidly increased in extent, remains constant in thickness except in the areas which later disintegrate in the formation of the perforations. These areas become thicker and marginally limited (Figs. 3.8 C). In section they are lens-shaped (in many herbs) or plate-like (in many woody plants) and can often be seen to be three-layered, consisting of the primary walls of the two adjacent cells and the middle lamella. The primary walls are chiefly of cellulose, with doubtless some pectic substances; the middle lamella is largely pectic. Multinucleate stages have been reported in the development of primary vessel elements in the Euphorbiaceae, but the uninucleate condition is apparently characteristic of all types of vessel elements.

Throughout the enlargement of the cell the cytoplasm remains abundant and active. As matuaity is reached, it begins a slow disintegration. In some woody plants the nucleus becomes small and greatly flattened and lies in scant cytoplasm against the wall where performation is about to occur (Fig. 3.8D); in other plants it maintains a more or less nearly central position in the cell.

After the primary wall is mature, and in some wood plants the secondary wall is partly, perhaps even fully formed the perforation of the end wall and loss of the protoplast begin. The wall in the perforation area becomes thinner, and, as the cytoplasm gradually goes to pieces, it also disintegrates—in same plants simultaneously throughout, in others beginning in the centre. The disappearance of this piece of wall is in some plants at first by a thinning, suggesting a dissolving, followed by a breaking up into several delicate layers (Fig. 3.8E). Statements that these walls are ruptured early in vessel development under tension as the cells rapidly increase in diameter and that the broken walls retract to form the rim are based on inaccurate observation.

Maturation does not proceed simultaneously in the members of a vessel series but progresses from one end to the other. The statement that a vessel matueres simultaneously from the base of a tree trunk to its tip is not brone out by careful study. The cambium, which forms the rows of vessel-element initials, itself progresses in activity from one region to another in various directions.

The progress of performation of vessel elements seems to be closely similar in the protoxylem and metaxylem of herbs and the secondary xylem of trees. It apparently has not been studied in detail elsewhere.

The Sieve Cell and Sieve Tube Element

Sieve cells and sieve tube elements are morphologically equivalent and are alike in fundamental structure and in function. They differ in that the perforations of the walls of the sieve cell and their cytoplasmic strands are all alike whereas those of the sieve-tube element are of two degrees of specialization, and in that sieve cells are not arranged in series as are sieve tube elements. These sieve cells and sieve-tube elements are elongate living cells with a thin cellulose wall. The protoplast has a large central vacuole and a thin peripheral layer of cytoplasm. No nucleus is present when the cell is mature. The cytoplasm contains leucoplasts which in some plants accumulate starch or similar substances. In some dicotyledons the vacuole contains slimy materials (of proteinaceous nature) which may be distributed throughout the cell sap or accumulate in masses in various places. The *slime plugs* seen in sections in some plants are doubtless artifacts, aggregations of slime caused by injury to the tissues. The wall apparently is composed of primary layers only. In some genera, for example, *Liriodendron*, it may become thick at some stages, perhaps serving then as a place of food storage.

Sieve areas and sieve plates. Sieve cells and sieve-tube elements are unique as living, functioning cells in the absence of a nucleus and the presence in the walls of fine pores through which extend strands of cytoplasm that resemble plasmodesmata but are commonly of much greater diameter and, unlike plasmodesmata, are sheathed with callus. The connecting strands of cytoplasm of sieve elements are clustered in areas of the wall known as *sieve areas*. These areas, which are unspecialized in gymnosperms and pteriodophytes, become highly specialized in angiosperms by enlargement of the strands and by sharper limitation. Such elaborated areas occupy more or less definitely restricted parts of the cell wall—usually of the end walls—which are known as *sieve plates*. Two types of sieve plates are distinguished—*simple*, with one sieve area; *compound*, with several sieve areas. In simple sieve plates the pores and strands tend to be very large, and the plate occupies all or nearly all of the end wall, which is commonly transverse. In compound sieve plates the pores tend to be smaller than those of simple sieve plates, and the plates occupy only part of the usually oblique end wall.

The sieve areas are scattered over the side and end walls or are in part or wholly restricted to certain walls, as to the radial and end walls in sieve tubes with long-tapering ends and to the end walls where the cell tapers more abruptly or has a transverse end wall (Fig. 3.9). The number of sieve areas on the side walls varies greatly; there are usually few or none where the end wall is transverse or nearly so (Fig. 3.9 P, S); where the end wall is long-tapering, the side wall—as well as the end wall from which it is hardly to be distinguished—may be completely covered by closely set sieve areas (Fig. 3.9 A, E). The number and position of sieve areas is to a large extent controlled by the position and arrangement of the surrounding sieve tubes. Sieve tube elements may be somewhat lobed or forked and may

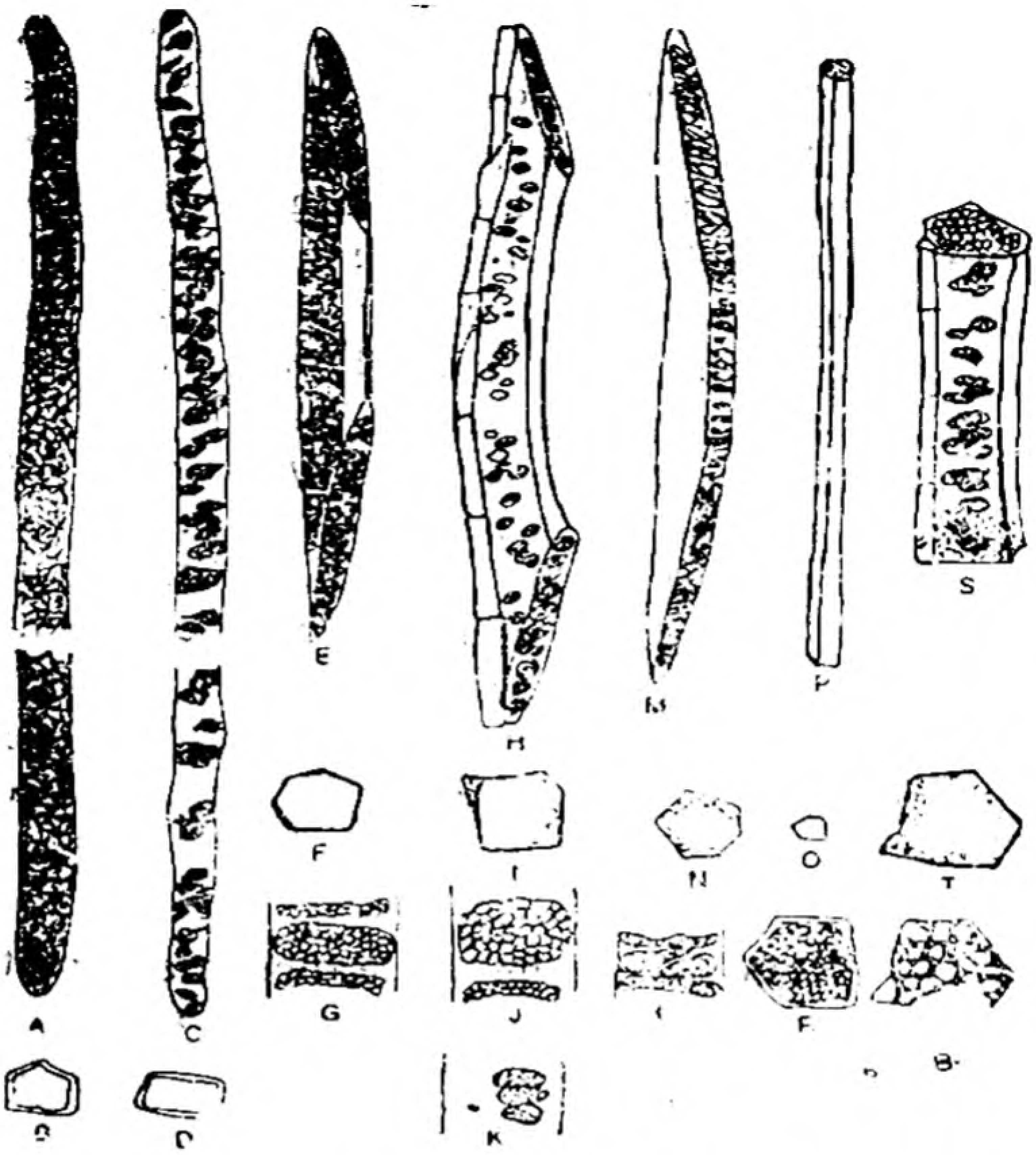


FIG. 3.9: Sieve cells and sieve-tube elements in side view and cross section, with detail structure of sieve plates. A, B, from Pteridium, only one-fourth of cell shown; C, D, from Tsuga canadensis, only one-third of cell shown; E.F.G, from Juglans nigra, G. part of sieve plate in detail; H, I, J, K, L., from Liriodendron—H, I, with companions cells attached, J, K, detail of sieve plate and of lattice respectively, L, sieve plate in section; M, N, O, from Molus pumila, O, detail of sieve plate; P, Q, R, from Solanum tuberosum, R, detail of sieve plate; S, T, U, from Robinia Pseudo-Acacia, with companion cells attached, U, detail of sieve plate.

even have three definite ends. Such cells stand at points of branching of a sieve tube. In phloem with tubes of the more primitive type such branching may be frequent, the tubes forming a loose mesh system rather than linear unbranched or rarely branched series, as in the highest type. Where well-marked sieve areas are lacking on the side walls, vestigial sieve plates, known as *lattices*, are often present (Fig. 3.9 H, S). These resemble typical sieve areas and plates of various types but are indefinite in outline and often ghost-like; the perforations are exceedingly minute, being usually no larger than normal plasmodesmata (Fig. 3.9 K) and often apparently lacking. Differences in the size of the pores in sieve plates and lattices of woody plants is shown by the following measurements: in *Juglans nigra* size of pores in the sieve plate is 1.8 to 3.5 micra, that in the lattices, 0.5 to 0.6 micra; in *Populus deltoides*, the sizes are respectively, 3.5 to 5.5 micra and 0.5 to 0.6 micra; in *Salix nigra* 2.0 to 3.0 micra and 0.4 to 0.5 micra. Stages intermediate between typical sieve areas and lattices may be found. Lattices are common in sieve tubes of intermediate type; they are usually absent in most herbaceous plants. The term "*sieve field*," sometimes applied to lattices, has become confused in use and is probably unnecessary.

Sieve tubes. Union of the sieve-tube elements to form a *sieve tube* is secured structurally by modification in shape and arrangement so as to form a linear tube and functionally by the greater specialization of the connecting strands in the end walls of the elements. In evolutionary development the sieve-tube elements have become increased in diameter and shortened. They have a range in shape similar to that of the tracheidvessel series: in the most primitive type the ends are long-tapering, and an end wall as distinct from a side wall is hardly apparent (Fig. 3.9A, C); commonly the well-defined end wall is oblique (Fig. 3.9E, H, M) or, in the most specialized forms, transverse (Fig. 3.9 P, S). Also in evolutionary development of sieve tubes their sieve areas decrease in number. The primitive type of sieve tube has many sieve areas on a plate occupying a long, oblique end wall, and these areas closely resemble the many areas of the side walls; the most advanced type has one area in a plate occupying hearly all of a transverse end wall, and areas on the side walls are scarce or absent. The diameter of the connecting cytoplasmic strands in this highst type is much greater than that of other types. The sieve tube type is not constant in families or sometimes even in genera. The primitive type is found in some families considered advanced, such as the Caprifoliaceae, and the most advanced type occurs in such primitive families as the Moraceae and Ulmaceae. Evolutionary advance in sieve-tube type has apparently taken place within families (Fagaceae, Rosaceae, Leguminosae) and within genera (*Fraxinus Prunus*). Although the sieve tubes of vines and herbs are usually of the highest, type, all types occur in woody and in herbaceous forms.

Ontogeny of the sieve elements. The mother cells of sieve elements vary in shape from short cylindric to elongate, slender, and tapering. Primary pit-fields are numerous on the walls, especially in secondary phloem, as on young tracheid walls. As these cells differentiate, they elongate; the cytoplasm becomes highly vacuolate and streams actively; the wall thickens; sieve areas develop from the pit-fields, and the cytoplasmic strands become prominent and increase in size; callus develops around the connecting strands. The relation of pit-fields to sieve areas in ontogeny is not altogether clear. Apprrently on pit-field may from one or more sieve areas, or several pit-fields form one area where the sieve plate is simple. In plates of this simple type when the connecting strands are very large, one or more pit-fields perhaps take part in the formation of the pore, each strand representing one greatly enlarged plasmadesmata thread or the fused threads of one pit-field. In the development of callus, rings are first formed about the strands at the apertures of the pores; increasing deposition forms cylinders that sheath the strands.

As the sieve tube element reaches maturity in size, the wall becomes thinner, the nucleus disintegrates, the connecting strands continue to increase in diameter, the streaming of the cytoplasm ceases, the periphepal layer of cytoplasm becomes extremely thin, the boundary between the cytoplasm and the vacuole becomes indistinct, and the semipermeable properties of the cell appear to be lost. At this stage the functioning period of the cell as a conducting structure apparently begins. In all plants conduction by sieve tubes probably lasts only a short time—from a few days in the early-formed primary phloem to a year or perhaps more in the secondary phloem of woody plants.

During the functioning life of the sieve tube, the callus increases in amount, lengthening the cylinders about the strands. Callus is deposited also on the wall around and between the strands forming with the cylinder a cushion-like mass over the sieve area. In the late stages of thickening in this cushion, the strands become attenuate, and some or all of them may be broken off. This condition seems to accompany the death of the protoplast. The callus cushion is for this reason known as the *definitive callus*. In some woody plants, where sieve tubes seem to function during a second growing season—perhaps even longer—the protoplast does not die, and the definitive callus is dissolved as activity in conduction is renewed. Under these conditions the sieve strands must remain unbroken. Little is known in detail concerning the structure of sieve tubes that appear to function through more than one growing season. Callus cushions forms only weakly or not all over the sieve areas of lattices. This with the presence of few weak connecting strands, or none, is evidence of the vestigial nature of these structures. Lattices form a transition stage in lateral wall structure between the numerous sieve areas of the primitive sieve tube element and the absence or great reduction in number of the highest type.

Obliteration of sieve tubes. After the loss of the protoplast of the sieve tube element the empty cell wall, at this stage as thin as in early stages of development, is crushed or collapses under the pressure and tension of the surrounding tissues which are brought about by increase in diameter of length of the organ of which they are a part. In many herbaceous plants the callus cushions and cylinders are still present when crushing takes place; in secondary phloem of woody plants they have disappeared. The crushing of the deal sieve tubes and their companion cells commonly becomes so complete that these cells are soon represented only by strands or sheets of structureless mrterial, and this material may be soon absorbed. In most plants, monocotyledons probably excepted, the surrounding living cells crowd into the space formerly occupied by the crushed cells, and evidence of the earlier structure of this tissue is difficult to find. The crushing and absorption of sieve and companion cell is known as *obliteration*.

Sieve cells of gymnosperms. Sieve cells have not been studied so intensively as sieve tube elements, and the structure of their sieve areas is more difficult to ascertain. The connecting strands are said to be arranged in small groups, and the callus cylinders appear to surround these groups rather than individual strands as in angiosperms. The history of development, functioning period, and obliteration is much the same as that of sieve tube elements. It is possible that the sieve cells of vascular cryptogams have a much longer functioning period—even several years—than those of seed plants.

Companion cells. The *companinon cell* is a specialized type of parenchyma cell which is closely associated in origin, position, and function with sieve-tube elements. These cells occur only in the angiosperms but in these plants accompany most sieve-tube elemements. Perhaps protophloem sometimes lacks them, and they appear to be scarce in the primary and early secondary phloem of some woody plants. In highly specialized phloem, such as that of many monocotyledons, they are abundant, together with sieve tubes making up the entire tissue.

Companion cells are formed by longitudinal, or oblique–longitudinal, division of the mother cell of the sieve tube element before specialization of this cell begins. One daughter cell may become a companion cell and the other a sieve-tube element; or the latter may divide further, forming more companion cells. Transverse divisions in the companion-cell initial may form a row of companion cells so that one to several may accompany each element. A companion cell or a row of a few such cells formed by transverse division of a single companion-cell initial may extend the full length of the sieve tube element. In a species the number of companion cells accompanying a sieve-tube element is fairly constant. Solitary, long companion cells are common is primary phloem and herbaceous plants; short and numerous companion cells appear to be characteristic of the secondary phloem of woody plants.

Companion cells have abundant granular cytoplasm and a prominent nucleus which is retained through the life of the cell. They do not contain starch at any time. They live only so long as the sieve tube element with which they are associated and they are crushed with those cells.

When seen in transverse section the companion cell is usually a small, triangular, rounded, or rectangular cell beside a sieve-tube element (Fig. 3.9 I, T). Often it seems to lie as if within the limits of the sieve tube element. If it does not, it may be impossible to determine with which particular sieve tube it is associated for it may be in contact with more than one. In secondary phloem of many gymnosperms the marginal cells of the rays, called *aluminous cells*, differ markedly from the other ray cells.

Phloem Parenchyma

Phloem typically contains parenchyma cells of other types than companion cells. Various terms have been given to these on a basis of shape and probable function, but these terms are confused in usage and the differences hardly merit distinction. In shape these cells range from elongate and tapering to broadly cylindrical, sub-spherical, and polyhedral (Fig. 3.10). The elongate cell, while young, may divide forming a row of cells that retain in their mature form and position evidence of this origin. In content also these cells show great variety: crystals, tanniferous substances, mucilage, latex, etc. Most of the parenchyma cells are filled with starch or oil during dormant periods. They remain alive until cut off from the inner living tissues by periderm formation. Those of secondary phloem in age may be transformed into sclereids, as in *Quercus*.

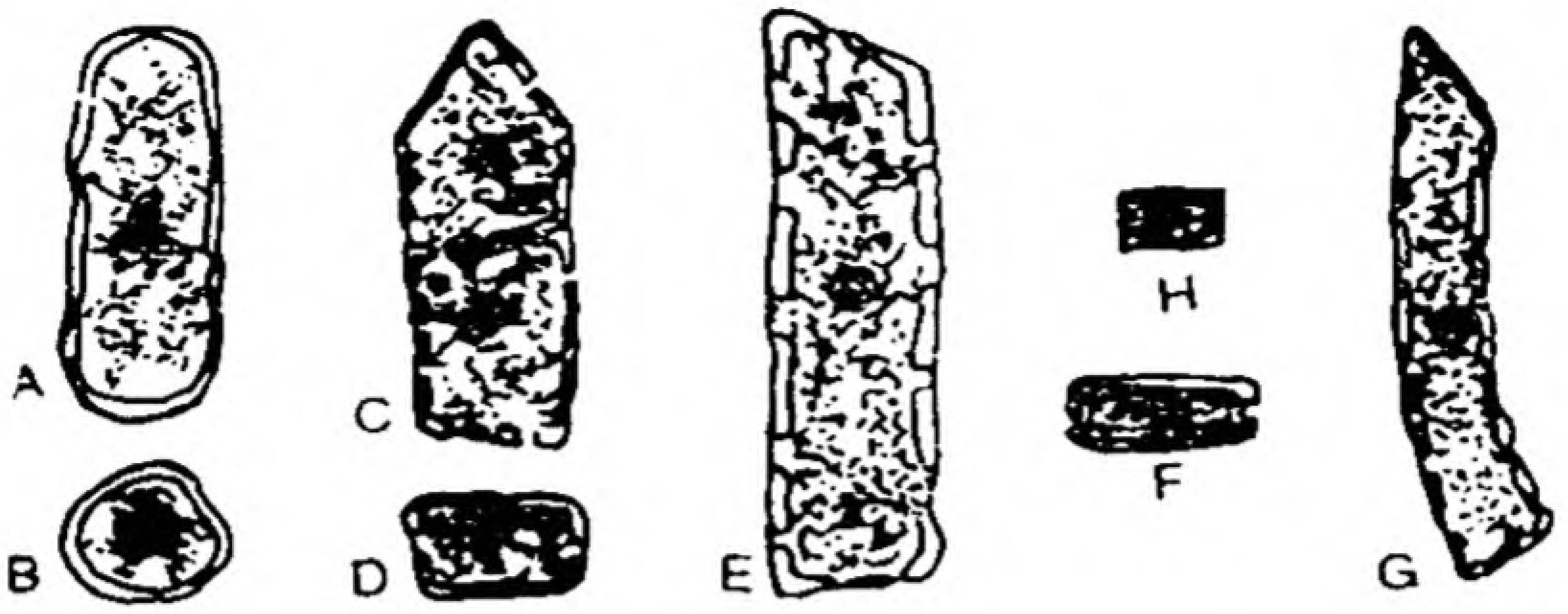


FIG. 3.10: Phloem parenchyma, longitudinal and transverse sections. A, B, from Salix nigra; C, D, from Robinia Pseudo-Acacia; E, F, from Liriodendron; G, H, from Malus pumila.

Parenchyma may be lacking in phloem—the tissue consisting of sieve tubes and companion cells only, as in the vascular bundles of many monocotyledons.

Phloem Fibres and Sclereids

Sclerenchymatous cells are rare or absent in phloem of living pteridophytes, and they are absent from this tissue in some gymnosperms and angiosperms. But in many seed plants, fibres form a prominent part of both the primary and the secondary phloem (Fig. 3.11). The fibres of the primary phloem—or part of these cells—have in some plants been mistaken for pericyclic fibres. Phloem fibres differ from xylem fibres in that the pits, which have small, linear, or rounded apertures, are always simple. The walls are lignified. In development the long-tapering ends become interlocked and strong strands are formed. The fibres often form tangential sheets or cylinders enclosing the inner tissues. These are obviously of structural importance as layers protective to the soft cambium region within, and also to some extent as longitudinal strengthening tissues. In some herbs and occasional woody plants, such as *Dirca palustris*, they apparently are of more importance in supporting the stem than is the xylem cylinder.

Fibres of the protophloem, forming the outermost cells of the primary phloem, are prominent features of many stems, both

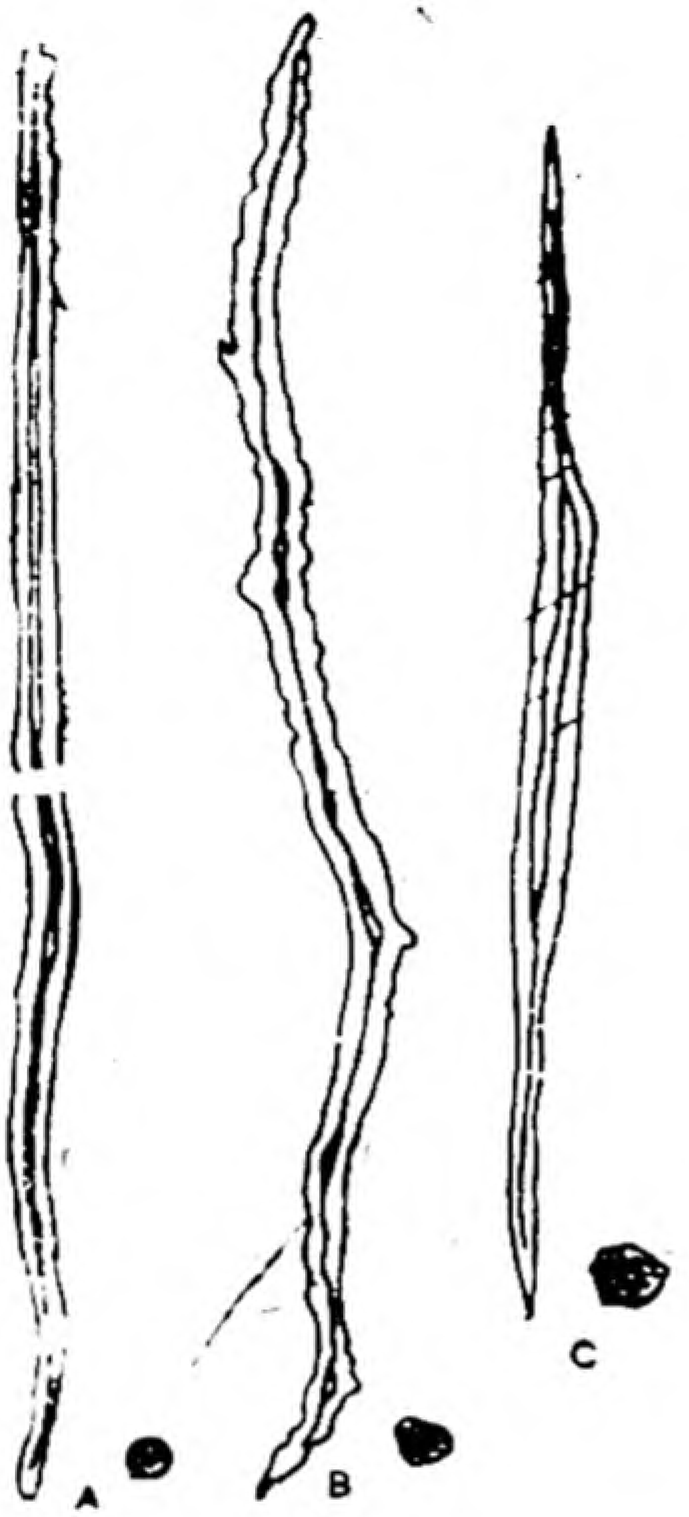


FIG. 3.11: Phloem fibres, longitudinal and transverse sections. A, from Salix nigra, only two-thirds of cell shown; B, from Malus pumila; C, from Robinia Pseudo-Acacia. (The scale is twice that of the figure of sieve elements, Fig. 3.9).

woody and herba ceous. In the early stages of sten development these fibres may contribute largely to the support of the stem. They are arranged in various ways: as continuous, uniform, or irregular bands; as scattered, isolated strands; as clusters "capping" the primary phloem strands. Such fibres are usually lignified, as are those of *Cannabis* (hemp), but may be of cellulose, as in *Linum* (flax). These primary phloem fibres are often similar to fibres of the cortex and to those of the secondary phloem. All or any of them with other fibres and sometimes vascular bundles constitute commercial "bast."

The term "bast."—Because of the strength of strands of phloem fibres, these have long been used in the manufacture of cords and ropes and in the weaving of matting and cloth. Fibrous tissue used in this way has been known since early days as *bast* or *bass*. The term was originally applied to any fibres obtained from the outer part of a plant, though a large part of such material came from the secondary phloem, as in the basswood, *Tilia*. When the secondary phloem was recognized as distinct from the cortex, the term "bast" was applied to this phloem, which was the common source of fibres. And in this sense, that is, as synonymous with phloem—as wood is synonymous with xylem—"bast" is still in frequent use. With the term used in this morphological senset the fibres of the phloem become *bast fibres*. But the term "bast, fibres," or simply "bast," is also applied to any fibres from the outer parts of a plant. This is topographical not histological or morphological use; such fibres may be a part of the cortex or pericycle. Secondary phloem is also frequently "divided into hard phloem. or bast, and soft phloem"; and "bark" is divided into "bast and living phloem." The term "bast" is used to such an extent without accurate botanical meaning that it should be discontinued as a technical term. Furthermore, it is superfluous, since the terms "phloem," "phloem fibres," "pericyclic fibres," and "cortical fibres" cover accurately all its uses.

Primary phloem occasionally contains sclereids, and the older secondary phloem of many trees contains abundant cells of this type. These cells develop from parenchyma cells as the tissue ages and the sieve tubes cease to function.

Pitting in Cells of the Phloem

In phloem the complex cellular relationship, the delicate walls of many of its cells, and the similarity of pits with plasmodesmata to sieve areas with cytoplasmic strands make the determination of pitting difficult. Statements concerning wall structure in some positions vary greatly, and much additional information is needed. The pitting of the thicker walled cells is clear: the pit-pairs are the simple pit-pairs of parenchyma and sclerenchyma, except that in some types of parenchyma the pits resemble sieve areas. Between sieve tube and parenchyma cells there is a sieve area on the side of the former and a pit on the side of the latter. Between the sieve tube and companion cell the wall is usually very thin and specialized areas are not seen; rarely, this wall is thick, and "pits" have been reported. It has been generally believed that companion cells are not pitted with parenchyma, but pit-pairs have been described in some plants.

Function of phloem. The chief function of phloem is the conduction of elaborated foodstuffs, both proteins and carbohydrates. The sieve elements are believed to be the cells concerned in this conduction with the companion cells, or the albuminous cells, related in some way to their activities. The sclerenchymatous tissues—libres and sclereids—serve or some measure in support of organs and protection of soft, underlying tissues. Many parenchyma cells are starch storage cells at certain periods; others perhaps serve in conduction of some substances; some are crystal storage regions.

The term "phloem." The term "phloem" is sometimes used—as is the term "xylem"—to indicate only the conducting cells of a complex tissue whose chief function is conduction. In this usage only the sieve elements of the tissue usually called phloem are indicated as "phloem." Occasionally all the "soft cells" are labelled "phloem," the sclerenchyma being excluded as "bast." The restriction of "phloem" to sieve tubes and companion cells is especially applied to protophloem. But histologically and morphologically the entire, continuous tissue is the phloem, and the term is so used in this book. For example: protophloem does not consist only of the sieve-tube elements that constitute its first-maturing cells but includes the much later maturing fibres that surround them.

"Transfusion tissue." A peculiar type of conducting tissue, which consists largely of short tracheids with thin, cellulose walls and bordered pits or reticulate or scalariform thickenings, often accompanies typical vascular tissue in the leaves of the gymnosperms. These cells are trached-like in the character of their pitting and in the absence of a protoplast but otherwise suggest elongate parenchyma cells. They lie adjacent to typical xylem at the sides of the bundle and may partly or even

completely surround it. Since these cells seem to serve as conducting tissue, connecting the veins and the mesophyll of theleaves, taking the place of the usual very small branches of the veins, they form together what is called *transfusion tissue*. Though the function of this tissue is still uncertain, it undoubtedly represents modified vascular tissue.

ARRANGEMENTS OF TISSUE

All the tissues of a plant which perform the same general function, regardless of position or continuity in the body, may be considered to form, together, a *tissue system*. In this sense the term is wholly a physiological one. There are found in physiological treatments of anatomy such tissue systems as the "mechanical system", the "absorbing system," and the "storage system." The various parts of most of such systems, however, are bound together only by function; they have little or no structural or morphological unity. Continuity and similarity of nature or of orign may be lacking.

From a morphological viewpoint the grouping of tissues into tissue systems is sometimes convenient. In the morphological sense, a tissue system must be a complex of cells extending continuously throughout the plant body or over a considerable portion thereof. It may be so simple as to consist of only one type of cell or one type of tissue or may consist of two or more types of tissues. Very few systems in any was structurally distinct can be distinguished. The older students of anatomy distinguished an *epidermal*, or *tegumentary*, *system*; a *fundamental*, or *ground*, *system*; and a *vascular system*. The distinction of these systems is not often made in present-day anatomy, doubtless because the systems have, in use, been morphologically too inclusive or indefinite or have, been nonmorphological. The epidermal system has sometimes included a hypodermis and other cortical tissues—even periderm; the fundamental system, the cortex, pericycle, and pith. Recognition of these systems has value in a strictly topographical sense and is especially useful in the interpretation of meristematic tissues. In young organs, the epidermal system consists of the dermatogen (or protoderm) alone; the vascular system consists of the procambium and the earliest formed xylem and phloem elements; the fundamental system consists of ground meristem—all the remaining tissues, which at this stage show little or no differentiation. In the mature primary body these form, respectively, the epidermis, the vascular tissues, and the cortex, pericycle, pith, and mesophyll.

The epidermis and vascular system are tissue systems of such uniformity and continuity of structure and of such constancy of function that they constitute important gross structural features of the plant body. The terms "epidermal system"—if used to cover epidermis only—and "vascular system" are convenient and valuable. "Ground tissue system" covers the heterogeneous remaining parts.

SECRETORY TISSUE

All cells directly concerned with the secretion of gums, resins, essential oils, nectar, and similar substances are together frequently referred to as "secretory tissue." Such a classification is purely a physiological one, as secretory cells and tissues often do not have common origin or morphological continuity. Secretory cells frequently are isolated from other similar cells, embedded in pith, xylem, phloem, cortex, or any region. On the other hand, secretory cells may be aggregated forming a tissue in the strict morphological sense. Not infrequently such cells constitute a definite, organized secretory structure or gland.

Secretory cells. Secretory cells are of two general types: those in which the secretion formed is exuded from the secreting cell, as in glandular hairs and secretory surfaces, such as nectaries and the epithelium of resin and oil canals; and those in which the secretion formed is stored within the secretory cell. The term *excretory cells* is often applied to the first type. This type of cell is usually characterized by a protoplast with richly granular cytoplasm and a conspicuous nucleus (Figs. 3.12 A, B, C, E, 3.13 B, C, D); the secretory cell is usually large with inconspicuous cytoplasm and large lumen filled with the secretion (Fig. 3.12 H). This type of cell contains in various

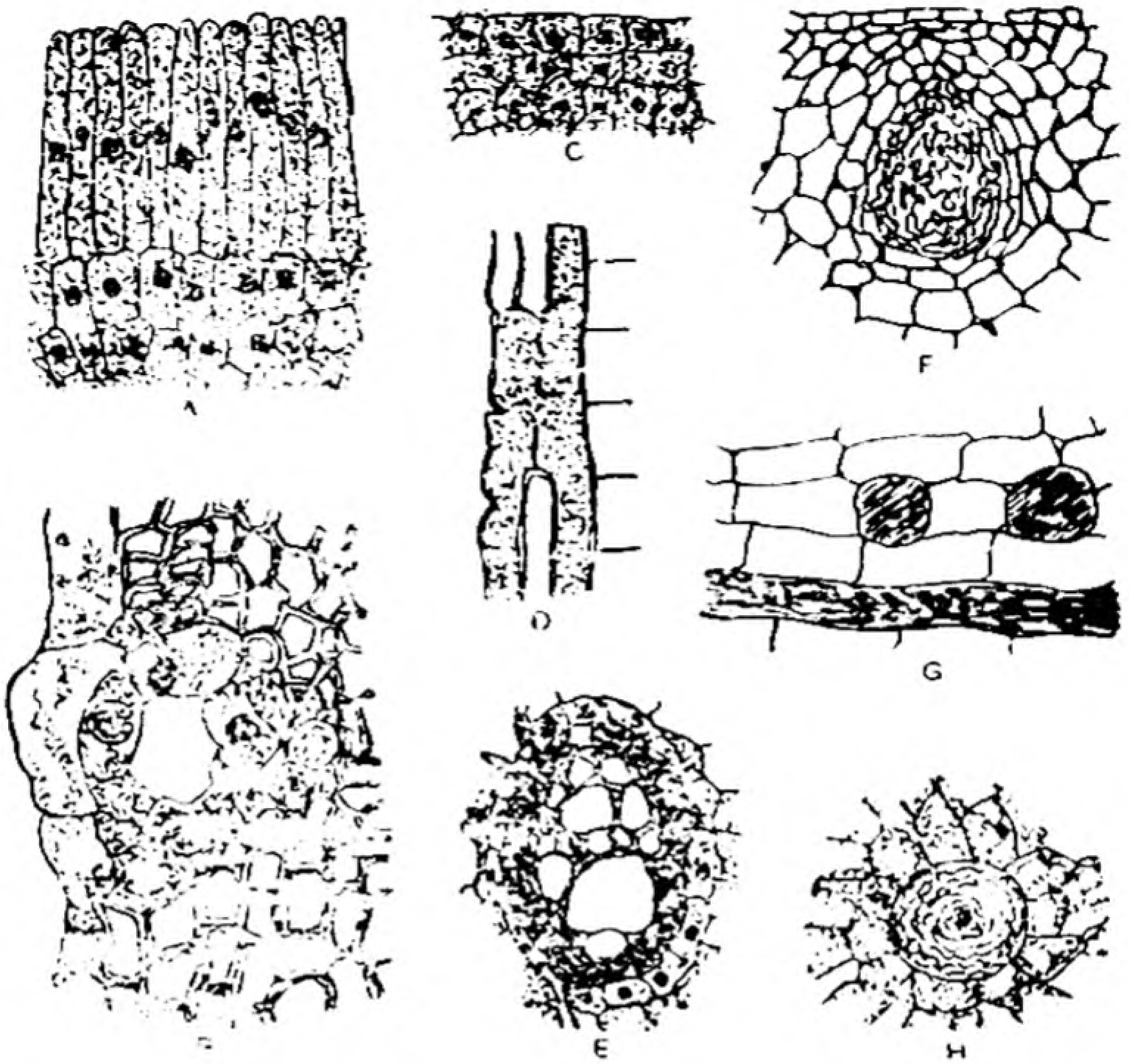


FIG. 3.12: Secretory tissue. A, section of surface of nectary of Euphorbia pulcherrima; B, cross section of resin canal of Pinus Strobus; C, section of floral nectary of Malus pumila; D, latex vessel from Tragopogon, absorption of cross-walls in progress; E, cross section of oil canal of young fruit of Angelica atropurpurea; F, section of lysigenous oil cavity of rind of Citrus sinensis; G, latex cells from cortex of Euphorbia splendens; H, secretory cell of bud scale of Liriodendron. (D, after Scott.)

plants many different substances, such as essential oils in *Liriodendron*, *Sassafras*, and *Zingiber*, and mucilage in many ferns. Glandular hairs often show elaborate specialization related to various functions, as, for example, the stinging hairs of the nettles, *Urtica* (Fig. 3.13 A). Such specialized hairs are often multicellular.

Glands

Glands are classified physiologically as either secondary as excretry, but it is difficult to draw a clear line between the two

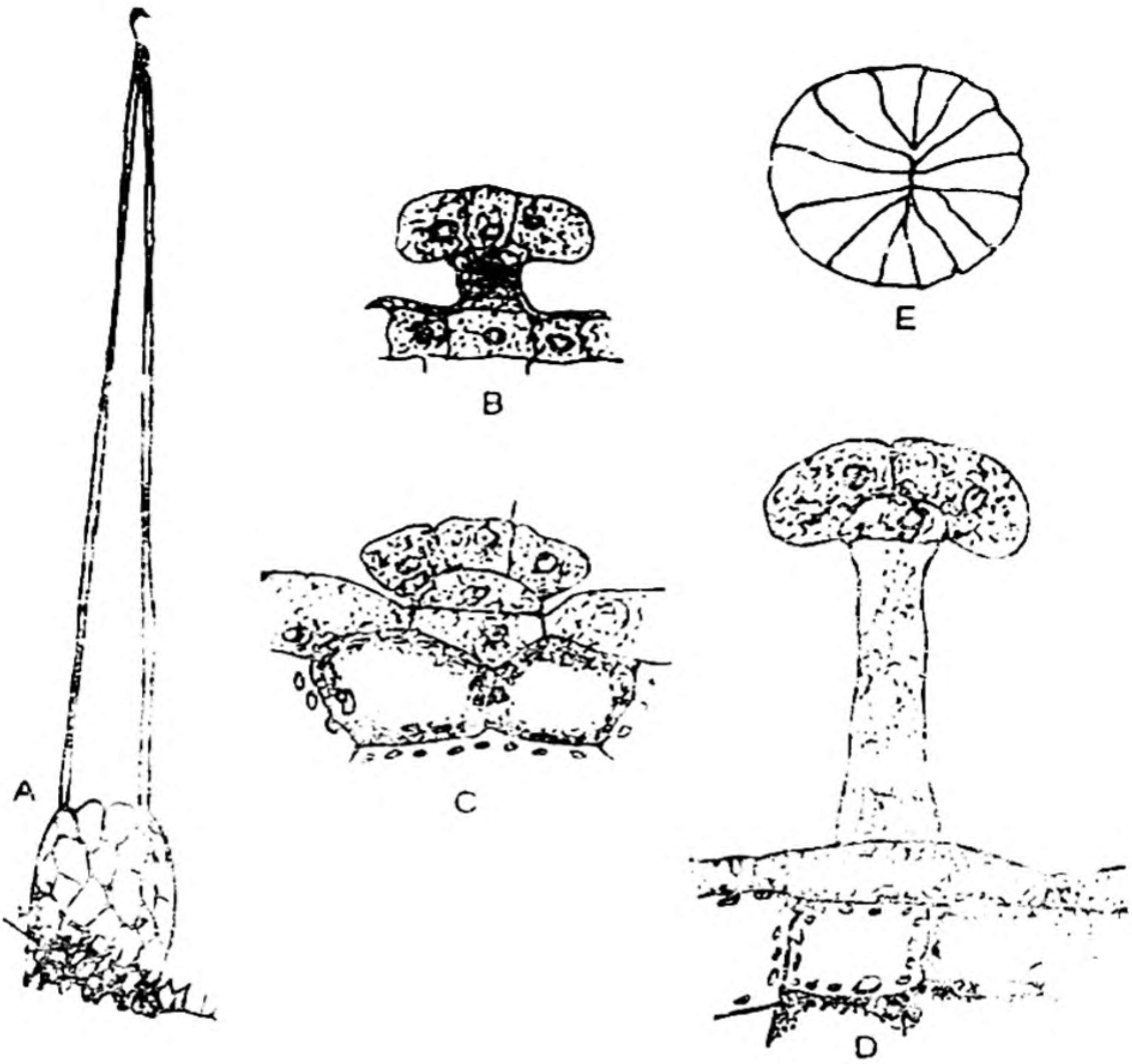


FIG. 3.13: Secretory hairs. A stinging hair from Urtica gracilis; B, glandular hair from ovary of Gaylussacia baccata: C, D, two types of glandular hairs from leaf of Pinguicula; E, top view of D. (The scale of A is much less than tnat of the others).

functions. Broadly speaking a secretion is directly valuable to the plant and an execution is a superfluous a deleterians substance. Plants have no regular method of evacyating their excretion as animals have. They dispose of them introductly. building thems into their structure usually in superannuated tissues. Thus they may sometimes become indirectly useful to the plant. For example, mineral materials may serve to strengthen tissues. Again, the aromatic terpenes, to which plant odours are due, are superfluous substances from the point of view of metabolism, but they serve an essential biological purpose in attracting insects to flowers, whereby pollination is effected.

From the anatomical standpoint it is simpler to classify glands as either superificial or internal. The superificial glands are usually modified hairs in which one or more cells contain the secreted material. A gland of this type is the stinging hair of the Nettle (*Urtica dioica*) (Fig. 3.14). The secretory cell is flask-shaped, the bottom of which is seated on a pedestal of small cells. The neck is drawn out into a long, tapering cone, the wall of which is impregnated with Calcium carbonate, and the tip is bent sideways and forms a small silicified bulb. When touched this bulb breaks off, leaving a sharp point, with a lateral opening like a hypodermal needle, which pierces the skin. Pressure forces the poison upwards from the flask-like base of the cell and into the subcutaneous tissues. The gland contains a mixture of histamine and acetyl choline. The former causes itching and the combination produces the prolonged burning sensation. Acetyl choline alone is without effect. ***Urtica stimulans***, the Java Nettle, causes serious illness, comparable in severity to a snake bite.

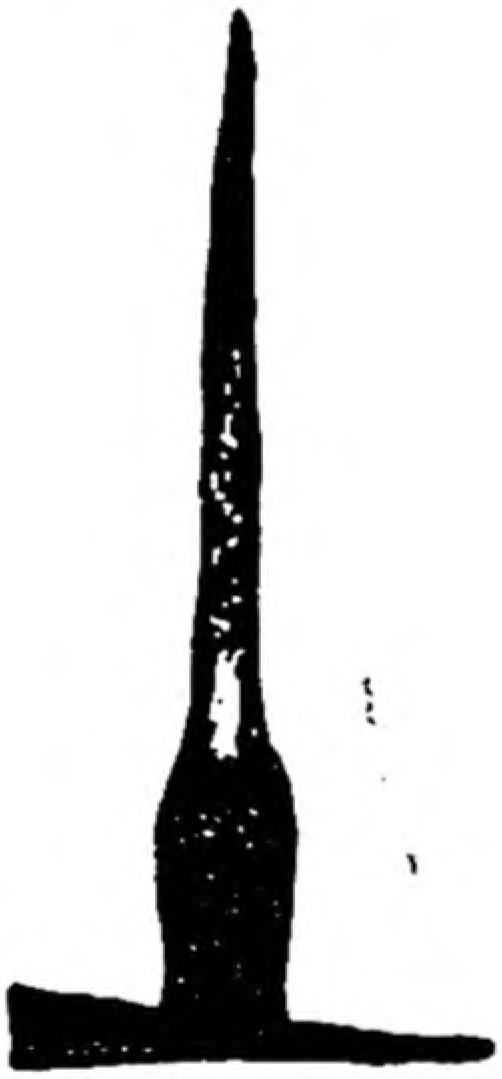


FIG. 3.14: Urtica dioica. Stinging hair showing pedestal of small cells, flask-shaped secretory cell with long neck and terminal bulb.

The glands produced on the leaves of the Insectivorous Plants are generally glandular hairs and they function both for the capture and the digestion of the insect prey.

Superficial glands, if not formed from hairs, may be simply modified patches of epidermis, as, for instance, in the *nectaries*, found in most flowers and sometimes also on leaves or stems, where they are called *extra-floral nectares*. The epidermis of these gland areas has no cuticle and consists of a layer of narrow, highly protoplasmic cells called *epithem*.

Digestive glands. In the majority of plants, enzyme secretion is not confined to specialized cells or tissues but is a characteristic of most living cells. In certain so-called "insectivorous" and "carnivorous" plants there are special glands which secrete protein-digesting enzymes; these enzymes act upon insects or other organisms so that the products of digestion can be absorbed by the plant. In *Droscra*, the secretory tissue is at the tips of the leaf "hairs" or "tentacles", structures which also serve to imprison the insects. Here, in addition to the digestive enzymes, there are secreted viscid substances which hold the insects. In such plants as *Nepenthes* and *Sarracenia*, which normally have pitcher-like transpartly filled with liquid, the glands are sessile and secrete the enzymes into the liquid from which the products of digestion are absorbed. In some other genera, for example, *Dionaea* and *Pinguicula* (Fig. 3.13 C, D), the glands are inactive except when stimulated by contact with animal matter. Less specialized glandular tissue of this type is found in the embryos of certain seeds, but such tissue is usually not clearly differentiated.

Nectaries. Many entomophilous plants produce nectar which attracts insects. This substance is secreted by specialized cells either on the floral parts themselves or, more rarely, on bracts or other structures outside the flower. Usually, the secretion of nectar is from specialized epidermal cells which cover certain regions of the flower, rather him from number the organs adapted to secretion alone. Definite and elavorate structures do, however, occur in certain families, for example, the Euphorbiaceae. In the less specialized nectaries the secreting cells are superficial upon the floral parts and in most plants closely resemble the other epidermal cells of the region but lack a cuticle (Fig. 3.12 C). Sometimes they are set off from the surrounding epidermal cells by a somewhat more columnar or papillose shape and by denser cytoplasm (Fig. 3.12 A). The nectar is exuded through the wall and exposed upon the outer or nectariferous surface. The secreting cells of stigmatic surfaces are of the same nature as those of nectaries but are often not clearly set off from normal epidermal cells.

The *septal nectaries* or *glands* of many monocotyledonous flowers are pockets in the septal walls of synearpous ovaries where the fusion of the carpel walls is incomplete and the epidermal cells are glandular. These glands may be simple, slit-like cavities or deep pockets with canal-like passageways to the surface of the ovary.

Hydathodes. Many plants possess structurally modified regions where water is exuded under conditions of low transpiration and abundant soil moisture. These are known as *hydathodes* or sometimes as *water pares* or *water stomata*. Morphologically, they are considered to be enlarged stomata which serve for water secretion. Structurally, they may resemble stomata closely but often they show elaborate structural specialization. Such structures do not "secrete" the fluid but merely provide and control the openings through which it escapes Hydathodes occur commonly at the tips of leaves as in grasses, at the apices of serrations on the margins of leaves, and in other positions. They are found mostly on plants of humid climates.

Resin, Oil, and Gum Ducts

In the gymnosperms generally and in many angiosperm families, resins, oils, gums, and other substances are secreted and conducted in ducts. In some plants, as in *Pinus*, these ducts or canals may form extensive systems extending both vertically and horizontally. In other plants the ducts may be local in occurrence and limited in extent, as in the fruits of the Umbelliferae. In *Pinus* and closely related genera, the resin ducts are schizogenous in nature, and when mature, have the structure of a tube with an epithelial lining (Fig. 3.12 B, E). The oil ducts of the Umbelliferae (Fig. 3.12 E) are of the same general nature. The secretory cells lining these cavities are thin-walled parenchyma with dense protoplasm. In general, these cells are elongate with the long dimension extending parallel with the long dimension of the duct. The substances secreted are various in nature and in some plants—for example the resin of *Pinus* and of *Agathis* (Kaurigum), and some essential oils—are of much economic importance.

Another type of gland is that found in the aim of citrous fruits (Fig. 3.12 F). Here there is a lysigenous cavity filled with essential oil and other substances that have been formed by the disintegration of the cells and as definite sometimes before the breaking down of the tissues. The origin of this secretion is not well understood. These glands are the source of the essential oils of lemon and orange.

Laticiferous Ducts

Latex is found in a considerable number of anginosperm families. This substance appears as a white, yellow, or reddish, sometimes slightly viscous fluid which has been shown to be an emulsion of proteins, sugars, gums, alkaloids, enzymes, rubber and other substances suspended in a matrix of watery fluid. Starch grains may be abundantly present. The function of latex is not well understood. It is apparently secreted by the cells in which it is contained, and is conducted by them throughout the plant body. The latex of some plants is of great importance, especially as a source of rubber (H*eeca*, *Ficus*, etc.) chicle (*Achras*), and papain (*Carica*), as well as for other substances. Laticiferous ducts are of two types—one known as *nonarticulate latex ducts* or *latex cells* and the other as *articulate latex ducts* or *latex vessels*. The function and the contents of the two kinds of ducts are essentially the same, but the morphological nature and development are different.

Nonarticulate latex ducts. These ducts are individual cells that extend as ramifying structures for long distances through the plant body (Fig. 3.15). The walls are soft ond often thick, and the cytoplasm contains great numbers of nuclei. These ducts arise as typical small meristematic cells among other promeristem cells. They quickly become elongate, and their tips keep pace in growth with the surrounding meristem, penetrating among the new cells. They branch and extend through all the tissues of the plant, even in some genera (*Cryptostegia*) through secondary xylem. Although the branches of a cell come in contact with those of other cells, no anastomoses occur.

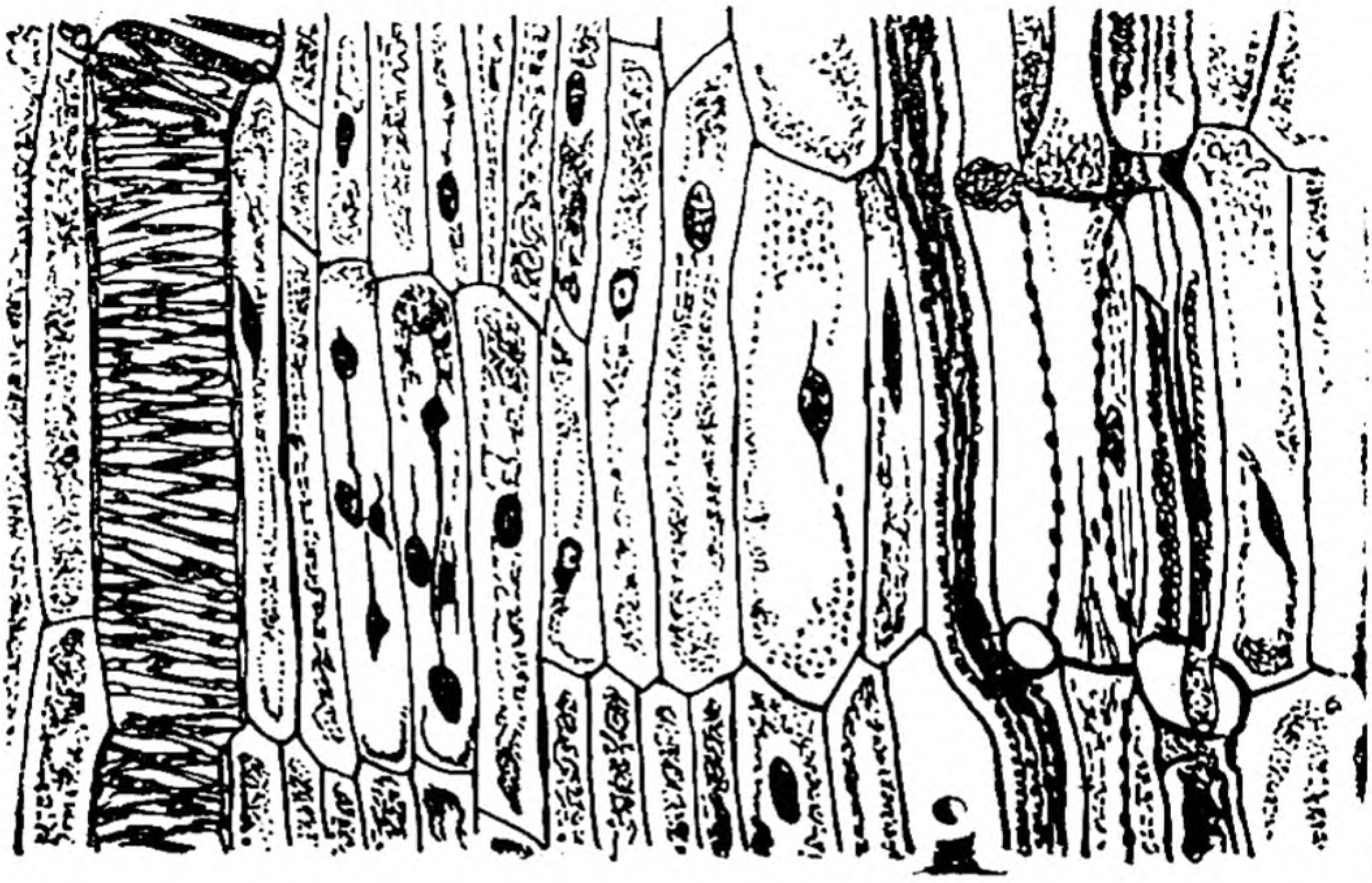


FIG. 3.15: Radial section through growing cambium region in root of Taraxacum kok-saghyz (Russian dandelion). At the left, a mature, scalariform protoxylem vessel with simply perforated, transverse end walls; in the center, cambium and its recent derivatives, two with cell division incomplete, the phragmoplasts migrating to the end walls; at the right, mature phloem consisting of sieve tubes, articulate latex ducts, and storage parenchyma. (After Artschwager and McGuire.)

Two kinds of these nonarticulate ducts are described but the differences lie in the number per plant, the position of origan and extend in the plant body rather than in structure or function. In one kind the cells arise only once, in small number, in the embryo. These few cells, extending with the meristems, ramify through the entire plant. The ducts of the Asclepiadaceae, most of the Apocynaceae and Euphorbiaceae, and those of some other families are of this kind. Ducts of the other kind arise repeatedly in the meristems and ramify into adjacent tissues but are restricted in their growth to one internode and attached leaf and branch. The ducts of the Urticaceae and those of some of the Apocynaceae (*Vinca*) are of this type.

Articulate latex ducts. These ducts originate in the meristems from rows of cells by the absorption, complete or partial of the separating walls early in the ontogeny of the cells. By branching and frequent anastomoses, a highly complex system is built up. A duct of this type resembles a xylem vessel in that it is made up of a series of cells united to form a tube by the dissolution of walls, but the latex tube is living and coenocytic. The Papaveraceae (poppy), Caricaceae (papaya), Compositae (dandelion), Musaceae banana), and the genus *Hevea* (Braziliar rubber tree) have this type of laticiferous ducts.

MERISTEM

The embryonic tissue which forms the *growing regions* of the shoots and roots is called *meristem* (*meristos*=divisible), because these are the sites of rapid cell-multiplication. A certain proportion of the cells produced, subsequently enlarge, cease to divide, and acquire thickened walls or undergo other changes and are then added to the *permanent tissues* of the plant. This change from meristematic to permanent form is called *cell differentiation.* A certain number of residual cells, however, always retain the meristematic character, so that meristem, in addition to forming permanent tissues, is also self-perpetuating. In most Pteridophyta the function of producing new cells is localized in a single *apical cell* instead of being distributed through a meristematic tissue. In this they resemble certain Thallophyta.

The tip of a stem is usually occupied by a bud, which is composed of immature leaves produced by the growing point itself. The latter lies in the centre of the bud, and a median longitudinal section will show that it is in fact the actual apex of the stem, which tapers rapidly within the bud, to end at this point. The meristem of the growing point is extremely soft, delicate and juicy, as seen with the naked eye. A similar tissue occupies the tip of the root, but no bud surrounds it in this case, because roots have no leaves.

Microscopically the cells of the growing point are small, fairly regular in size and shape and have thin, delicate cell walls formed of a mixture of cellulose and protein, with an intercellular layer of mucilaginous pectin. They have prominent spherical nucles, which are very large relative to the size of the cells, and the cytoplasm is dense and completely fills the cells. When a meristem cell divides, each half quickly grows to the size of the original cell, thus doubling the amount of protoplasm. It follows that it is here that the chief synthesis of protoplasm goes on. The cells fit closely together, without intercellular spaces and their shapes are those of plastic spheres in mutual compression, due to their active growth. They are either dodecahedra (twelve sided) or tetridecahedra (fourteen-sided). The youngest cells have no vecuoles and they never possess chloroplasts, though transitory starch grains may sometimes appear.

Opinion varies as to the exact limits of the term meristem. Some confine it entirely to the non-vacuolated cells, but there is a considerable interval between the first vacuolization of the cell and its completed differentiation, and many prefer to extend the term generally to all those cells which continue to divide, even when somewhat altered from their youngest form.

The meristem of the growing point is called the *apical* *meristem*, and the term *primary meristem* is applied both to it and to all meristem tissues derived directly from it. Thus the cambium in Dicotyledons and the meristems of lateral shoots are also actually primary meristems. In many Monocotyledons there are *intercalary meristems* at the bases of levels, and similar embryonic regions occur in older parts of Dicotyledons. All these are primary meristems. True *secondary meristems, i.e.*, those formed from tissues previously differentiated, are rare, the chief example being the cork cambium.

When we consider that the development of the meristem cells is responsible for determining the specific form of the plant and the structural anatomy of the older portions, it is obvious that it is the centre of many activities of great interest, about which we know very little. The evidence seems to show, however, that almost without exception the divisions of meristem cells are equational, and therefore that the potentialities of the cells produced are equal. There is little sign in plants of the primary segregation of germ cells and soma cells which Weismann suggested in animals. Theoretically every cell of the plant is *totipotential*, that is, it retains in itself the possibility of regenerating the whole plant, in suitable circumstances. Any actual limitations of this power are imposed by special conditions. The differentiation of cells is therefore explicable, not by inherent differences between them but by the influence of their environment, that is to say by differences of water and food supply, oxidation, etc., due to the position of the cell in the tissues.

Even within the meristem some differentiation is apparent. In 1868 Hanstein named three zones, which he considered to be typical and which he called *histogens* or "tissue producers" (Fig. 3.16). The outermost was a single layer of cells named the *dermatogen*, which gave rise to the epidermis. Beneath this came an intermediate zone, the *periblem*, giving rise to the cortex and the inner tissues of the leaf. Centrally was the core or *plerome*, producing pith and vascular tissues. In cases where such layers can be seen these cames still have a descriptive

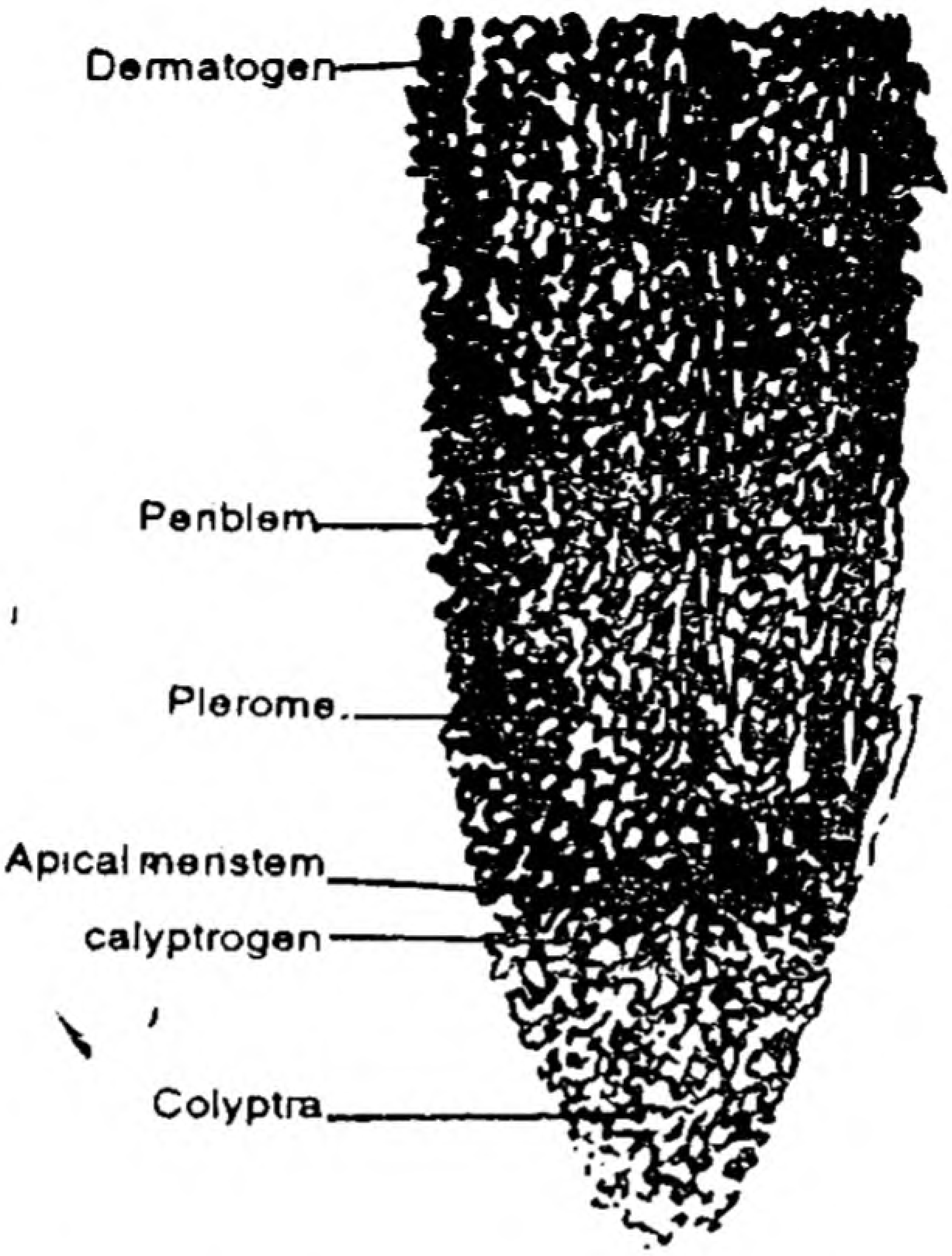


FIG. 3.16: Vicia faba. Longitudinal section of root apex showing the histogens.

value and are frequently used, but the developmental significance assigned to them by their author is not consistent with fact. Frequently, indeed, the three zones cannot be distinguished, especially at the apex of the stem.

Modern studies have interpreted the meristem of stems as composed of two zones only. These are the *tunica,* which consists of one or several peripheral layers of cells, enclosing the *corpus* or central core of tissue. The distinction between them reflects two different modes of growth. In the tunica, surface growth predominates and cell divisions are uniformly anticlinal, that is, perpendicular to the surface, with the result that the cells are regularly oblong in longitudinal section and each tunica layer remains distinct. In the corpus, by contrast, volume growth is predominant and the planes of cell division and cell arrangement tend to be highly irrgeular. The maintenance of balance between surface and volume growth leads to constant adjustments which find their expression in the rhythmical alternation of minimal and maximal areas of the growing point, the latter being associated with the formation of external folds or leaf primordia. These zones are not "histogens" in Hanstein's sense but represent the distribution of growth patterns, which may vary considerably in different species.

Immediately below the corpus may often be seen a tissue which is called the *rib meristem*, distinguished by short, vertical rows of cells in process of vacuolization, These are formed from cells of the corpus which have repeatedly divided horizontally. As each row is produced from one original cell it has a common wall surrounding it. This tissue continues downwards into the pith.

CLASSIFICATION OF MERISTEMS

Meristems are classified an several bases: stage of development, structure, position in plant, origin, function, topography. The bases of these classifications are not mutually exclusive, and the definitions cannot be rigid. A discussion of the more important types of meristems follows.

Meristems Based on Stage or Method of Development

Promeristem

The region of new growth in a plant body where the *foundation* of new organs or parts of organs is initiated constitutes *promeristem*. (The terms "primordial meristem," "Urmeristem," and "embryonic meristem" have also been applied to this initiating region.) Structurally this region consists of the initials and their immediate derivatives, all young cells with diameters much alike, walls thin, with early stages of pits; cytoplasm active, vacuolate or nonvacuolate; nuclei large; and intercellular spaces absent or minute. The promeristem of a given organ or region is of rather limited extent, varying in amount in different plants, in different organs and under differing growth conditions. It is of course not definitely set off from the somewhat older meristematic tissue into which it merges. A soon as cells of the promeristem begin to change in size, shape, and character of wall and cytoplasm, setting off the beginnings of tissue differentiation, they are no longer a part of typical promeristem; they have passed beyond that earliest stage. For example, there is at the tip of an organ a meristem of some length, only the youngest part of this, a small apical portion, is promeristem. The remainder of the meristem represents the early stages of the tissues formed by the promeristem. No term exists for this partly developed region in which segregation of tissues is beginning, but cell division continues freely.

Mass, Plate, and Rib Meristems

On the basis of plane of division, *mass*, *plate*, and *rib meristems* have been distinguished as "growth forms" of meristem. In mass meristem, growth is by three-plane or all-plane division and produces increase in mass; plate meristem is by divisions—chiefly in two planes—so that there is plate-like increase in area; rib meristem, by continuing divisions (anticunal) in only one plane, produces rows or columns of cells, functioning chiefly in the increase of organs in length. Examples of mass meristem are early stages of many embryos; developing sporangia; the endosperm of many plants; young pith and cortex of some plants. Plete meristem (one-layered) forms epidermis, and (two-to several-layered) is prominent is leaf development where in early stages divisions in the plane of the leaf blade and at right angles to that plane build up great increase in blade area with little increase in thickness. Rib meristem plays a prominent part in the development of young roots and of the pith and cortex or young stems. The term "rib" meristem is in frequent use at present but is unfortunately often applied not to regions of cell initiation but to those of cell differentiation where the cells stand in long rows. In general, these "meristems" are merely parts of meristematic tissue-masses distinguished by planes of cell division. Recently the term *file meristem* has to some extent replaced rib meristem; this is a more accurately descriptive term. It is obvious that these types can be distinguished only when the cells are chiefly of typical parenchyma-cell form; meristems with cells of prosenchymatous form cannot be thus classified. Cells that are beginning to elongate constitute the first stages of *procambium*, of *collenchyma*, or of strands of fibres.

MERISTEMS BASLD ON HISTORY OF INITIATING CELLS

Primary and Secondary Meristems

On the basis of type of tissue in which origin occurs, meristems are classified as *primary* and *secondary*. *Primary meristems* are those that build up the fundamental (primary) part of the plant and consist in part of promeristem. (In some uses, promeristems are considered distinct from primary meristems.) In primary meristems, promeristem is always the earliest stage, and transition stages to mature tissues constitute the remainder of the meristems. The possession of promeristem continuously from an early embryonic origin is characteristic of primary meristems; no stage wherein all or some of the cells have become permanent, or modified from the meristematic, has entered their history. The chief primary meristems are the apices of stems and roots and the primorida of leaves and similar appendages.

Exceptions to the promeristem continuity of primary meristems from early embryonic tissue are found in adventitious buds and roots and in some types of wound tissue. (Many so-called "adventious buds" arise from buried dormant bud intials, not *de novo* in permanent tissue.) The originating meristems of true adventitious organs arise secondarily in more or less nearly permanent tissues, but they are, because of their structure and behaviour, primary meristems. Once established, they may persist indefinitely.

Secondary meristems are set apart from primary meristems in that they always arise in permanent tissues; in their history there is interposed a stage of permanent tissue or at least of partial development toward permanency. They have no typical promeristem, though their initiating layers may to some extent resemble this tissue. *Secondary meristems* are so called because they arise as new meristems in tissue which is not meristematic. The cork cambium is an example of secondary meristem; it is formed from mature cells—cortical, epidermal, or phloem cells or from any mature cells that are not too highly-specialized.

The primary meristems build up the early and, for a time, structurally and functionally complete plant body. The scondary meristems later add to that body, forming supplementary tissues that functionally replace or reinforce the early formed tissues or serve in protection and repair of wounded regions.

The cambium, one of the most important meristems, does not fall definitely in either group. It arises from apical meristem of which it is a late and specialized stage. (Accessory cambia may be secondary.) The tissues formed by the cambium are secondary, as are all those formed by secondary meristems, whereas other primary meristems form only primary tissues.

The classification of meristems as primary and secondary has sometimes been considered of little value but it is especially helpful in an understanding of the manner in which the complex mature plant body is attained and modified with continuing growth.

Meristems Based on Position in Plant Body

On the basis of position in the plant body, meristems are usually classified as *apical*, "*intercalary*," and *lateral* (Fig. 3.17). Apical meristems are those which lie at the apices of the axis and of the appendages and are commonly called growing points. "Intercalary meristems" are those which lie between regions of permanent tissue, as, for example, at the base of the leaves of many monoeotyledons. Lateral moistems, as the name implies, are situated laterally in an organ. The cambium and the cork cambium are lateral meristems.

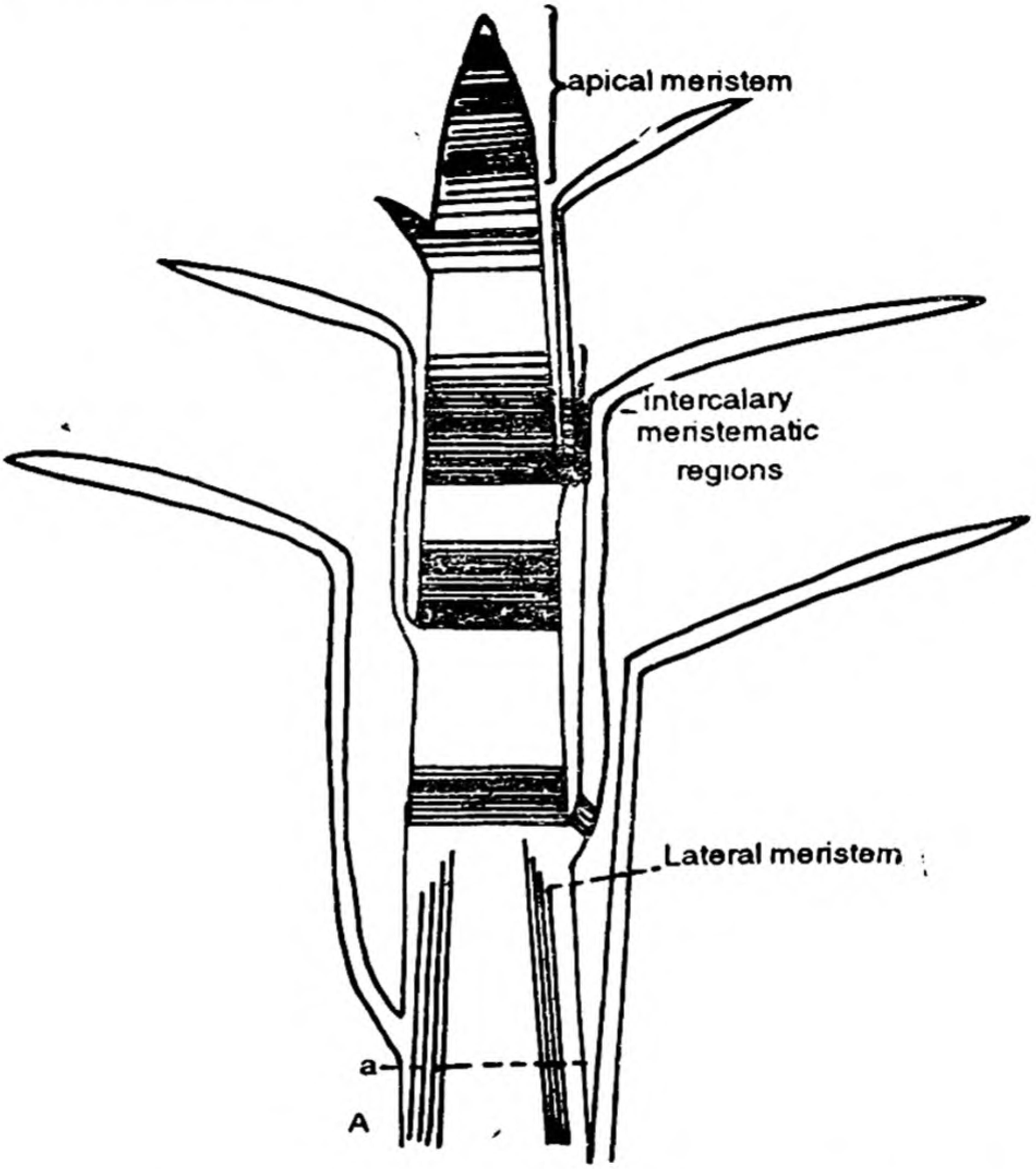

apical meristem
intercalary meristematic regions
Lateral meristem
a
A

FIG. 3.17: Diagram to show position of meristems. The closely lined areas are youngest; the unlined are mature of slowly growing. A, longitudinal view.

Apical Meristems

Apical meristems, or growing points, occur universally at the tips of the roots and stems, and often of the leaves, of vascular plants. The activity of these meristems brings about increases in the length of these organs, laying down the primary body of the plant. Initiation of growth is by one or more cells situated at the tip of the organ which maintain their individuality and position and are known as *apical initials* or *apical cells*. These cells may be strictly terminal or terminal and subterminal.

Apical cells. Among vascular plants, solitary apical cells (except those of leaves) occur in the horsetails, most of the ferns, and a few other pteridophytes. In other vascular plants a group or groups of apical, or apical and subapical cells constitute the initiating body. The behaviour of these cells in tissue formation has not been extensively and critically studied until recent years and is even yet only partly understood. Since the initials apparently differ little, if at all, from their recently formed daughter cells and since all of these cells may continue to divide freely it is most different to determine the number and limits of the initials. The number of initials is probably fairly constant for a given organ and a given species, but it is probable that at least in some plants, the number of initials and, to some extent, the form of the group vary from time to time even in the same organ. Meristems are highly plastic. Variations are related to vigour of growth, to seasonal conditions, and to the morphology of the organ developing. It is now known that in seed plants there are many types of apical-meristem differentiation. For each major plant group there is apparently a fairly characteristic plan of development, but this plan may not be consistently followed in detail even within an individual plant.

Initials, where solitary, appear to persist indefinitely; where there are several or many, they may function for a time and then be replaced by new cells. During periods of rapid growth, additional cells may serve more or less temporarily as initials.

Types of apical cells. Solitary apical cells of several types are distinguished by shape and by the number of sides from which new cells are cut off. The two most common are the lenticular, or two-sided cell (not found in vascular plants), and the pyramidal, or three-sided cell (Fig. 3.18). The former is strictly a three sided cell in shape and the latter a four-sided cell. but new cells are formed only from two and three sides respectively, hence terms have been given which apply to the activity of the cell rather than to its shape. The side from which no new cells are formed lies in the direction of growth. In roots, cells are cut off on this face also. The size of the apical cell is reduced only temporarily by the formation of daughter cells, and its position is maintained indefinitely.

The behaviour of solitary apical cells in segmentation, and of the cells which are cut off by the apical cells, is of importance in the study of the morphology, both vegetative and reproductive, of the bryophytes. In these plants, groups of cells in certain positions and of certain origin always form the same tissues or organs. That this must also be true in general of vascular plants was formerly believed, but such is clearly not the case. The behaviour of a given segment of an apical meristem may have little morphological significance in the groups of plants above the bryophytes.

"*Intercalary meristems*". So-called "intercalary meristems" are merely portions of apical meristems that have become separated from the apex during development by layers of more mature or permanent tissues and left behind as the apical meristem moves on in growth (Fig. 3.17). The more mature layers are the nodal regions and the "intercalary meristem" are therefore internodal. At early stages the internode is uniformly meristematic; later, some, part of it matures more rapidly than the rest, and a definite sequence in development within the internode is set up and maintained. The region of youngest cells is in most plants at the base of the internode, but it may be in the middle or at the top. Where the youngest region is basal, the order of maturing in the tissues of the internode is of course acropetal, where it is at the top, the order is basipetal; and where it is median, the sequence is both upward and downward.

It has been generally believed that "intercalary meristems" are separated from the mother meristem at so early a stage that they retain a layer of the promeristem of the apical region and that this promeristem continues, after its segregation, to function as a promeristem, initiating complete new segments of the organ. But it has been shown that this is not true in some grasses, and it is perhaps not so in any plants. Although these regions consist partly of promeristem cells, vascular strands, with protoxylem and protophloem cells in various stages of maturation run through them. With such structure they are not typical meristems but meristematic regions and should be so called.

The best known intercalary meristematic regions are those of the stems of grasses and other monocots and of horsetails, where they are basal, and those of some mints, where they lie just below the node. In some peduncles, such as those of *Taraxacum* and *Plantago*, they are said to occur at the top. Leaves of many monocotyledons (grasses, *Iris*) and some other plants, such as *Pinus*, have basal meristematic regions. Intercalary meristematic regions ultimately disappear; they become wholly transformed into permanent tissues.

Lateral meristems. Lateral meristems are composed of initials that divide chiefly in one plane (periclinally) and increase the diameter of an organ. They add to existing tissues or build new tissues. The cambium and the cork cambium are meristems of this type. The former has been called "the lateral meristem", but phellogen layers also belong here. The briefly functioning "marginal meristems" of some leaves have been called lateral but fall in none of the above groups.

MERISTEMS BASED ON FUNCTION: THEORIES OF STRUCTURAL DEVELOPMENT AND DIFFERENTIATION

The progress of differentiation in meristems brings about in cellular organization a structural segregation, or zonation, with regions disinguishable by:

(a) the number and position of the initiating cells,

(b) the planes of division and consequent arrangement of cells,

(c) the size, shape, and content of the cells, and

(d) the rato of maturation of the cells.

Several theories dealing with the methods of origin of the patterns formed by this zonation and their histological and morphological significance have been proposed.

The Apical Cell Theory

Solitary apical cells occur in many of the algae, in the bryophytes, and, among vascular plants, in the Psilotaceae, the horsetails, most of the ferns, and some species of *Selaginella*. Early studies of the apical meristems of bryophytes and of the pteridophytes with a single apical cell demonstrated morphological significance in the segmentation of the apex. It was believed that the same conditions, although difficult to demonstrate in seed plants, held for all higher plants, and the *apical cell theory* was proposed as the basis for an understanding of the method of growth and morphology in all groups. But controversy arose over the interpretation of the complex apices of gymnosperms, and it became evident that the theory was not applicable to seed plants.

The Histogen Theory

As a basis for the interpretation of the growing points of seed plants, the *histogen theory* replaced the older theory. Under this theory the more or less distinct major regions of the stem and root apex were called *histogens*, that is, tissue builders, in the sense of builders of specific parts of an organ. The histogens were the *plerome*, a central core; the *dermatogen*, a uniseriate, external layer; the *periblem*,* the region between the plerome and the dermatogen (Fig. 3.19). The histogens, or their individual layers, were believed developed by separate initials. This theory, in contrast to the apical cell theory, placed the origin of axis apices in a group of initials. It differed from the earlier theory also in morphological interpretations or implications—the plerome formed the pith and primary vascular tissues; the dermatogen, the epidermis; the periblem, the cortex.

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* These terms were originally applied to the zones of meristomatic tissue in early ges of development from initials but they have been occasionally applied to the tials only.

The viewpoint and the terminology of the histogen theory have long dominated anatomical interpretation of tissues, regions, and even organs in vascular plants. But the distinction of these histogens in an apex cannot be made in some plants, and in others the regions have no morphological significance. The plerome may form only the pith or the entire central cylinder and part of the cortex; the periblem may form the cortex and the outer stelar tissues or part of the cortex. Even in homologous axes on the same plant the histogens may form different parts.

The terms clearly have no morphological value, and, because their prominence has rested largely on such value, they are becoming less used. They are found occasionally in histological descriptions of stems, commonly in these of roots, but serve chiefly to indicate regions in a rather loose or topographical sense.

The Tunica-corpus Theory

Interest in the development of seed-plant axes reawakened about twenty years ago with the formulation of the *tunica-carpus theory* and increasing attention has been given to the ontogeny of the stem tips of vascular plants. According to the tunica-corpus theory (which has been applied only to the leafy stem, or shoot) different rates and method of growth in the apex set apart two regions of unlike structure and appearance a central core, the *corpus*, and an outer enveloping layer, around and above the corpus, the *tunica*. In the corpus the cells are large, with arrangement and planes of cell division

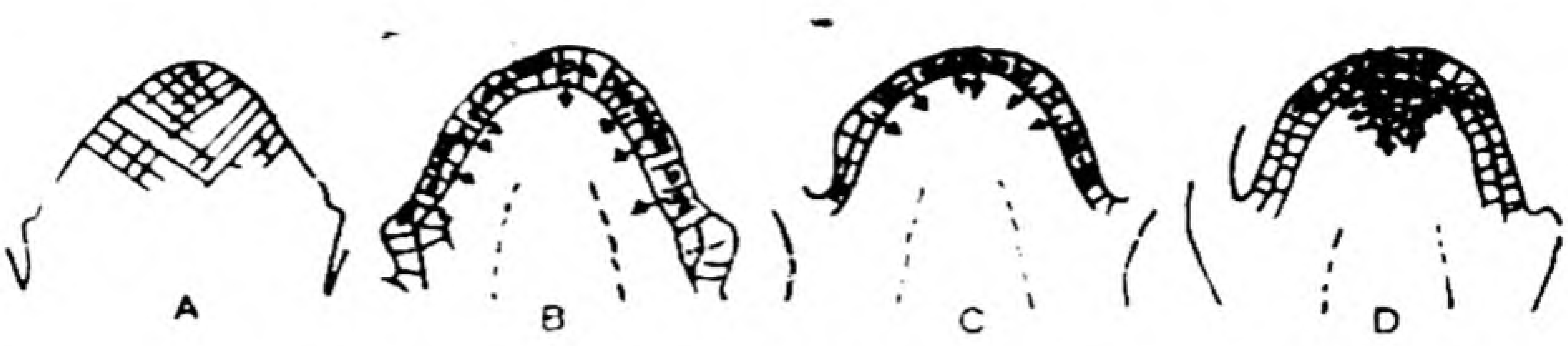

FIG. 3.18: Diagrams to show position and planes of division of stem-apex initials. **A**, initial solitary, with oblique anticlinal divisions only; **B**, initials many superficial, with their divisions and those of the dermatogen both anticlinal and periclinal; **C**, initials several, superficial, with their divisions both anticlinal and periclinal and those of the dermatogen largely anticlinal; **D**, initials in three tiers, the two outer, with divisions anticlinal only, forming a two-layered tunica and the innermost, with divisions in all planes, forming a corpus. (Initials indicated by outlined nuclei).

irregular, and increase is in volume; in the tunica the cells are usually smaller than those of the corpus and lie in layers or sheets, with divisions strictly, or chiefly, anticlinal, and growth is primarily in area. These regions vary greatly in the distinctness of their limits and in their form and relative size; their boundaries are often not clear-cut; the corpus may be massive or slender; the tunica, many-, few-, or one-layered. The important elements in such an elaboration of regional development are the position and number of initials and the planes of division in these cells and their derivatives.

The number of initials is few to several or many. Rarely, in small, very slender apices, such as those of grass seedlings, there may be only one or two in the tunica and about two in the corpuls. The determination of initials as self-maintaining and persistent cells is often impossible because they differ little or not at all from their recently formed daughter cells. Certainly it is unlikely that the initials remain constant in number, form, and sequence of division.

Where all the initials are on the surface, their divisions are both anticlinal and periclinal, and no clearly defined tunica and corpus are formed (Figs. 3.18A B, C, 3.20 A, B 3.21 A, B).

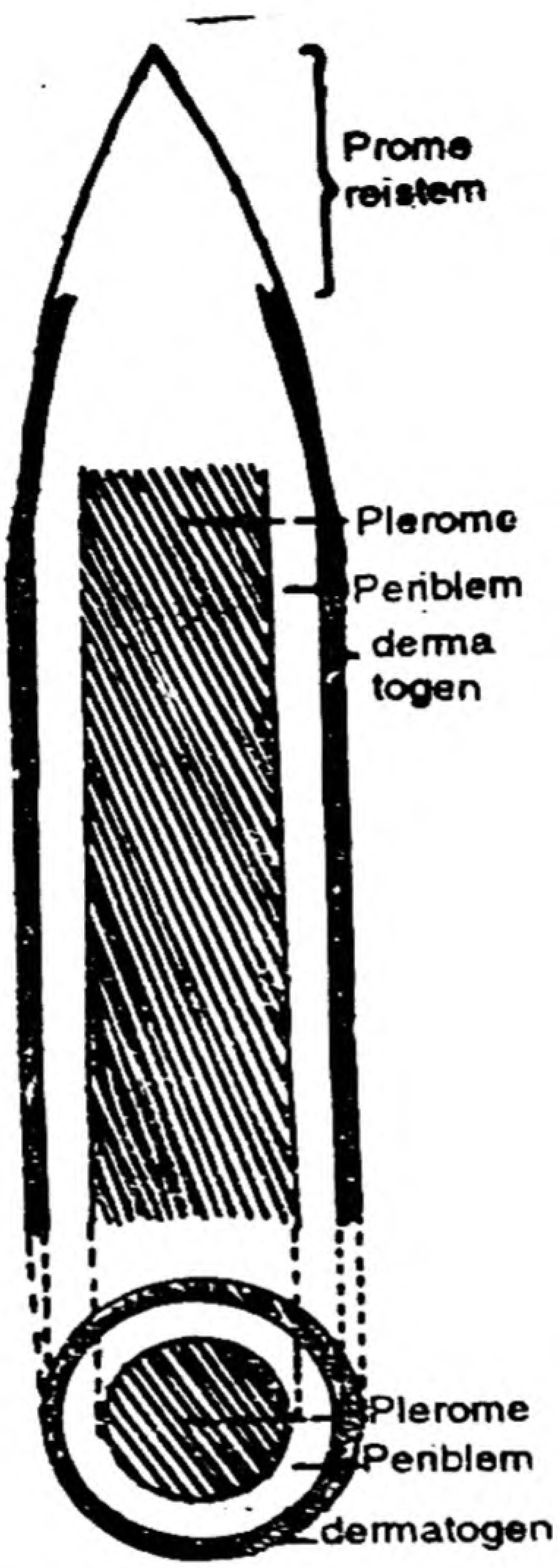
Prome
reistem
Plerome
Periblem
derma
togen
Plerome
Periblem
dermatogen

FIG. 3.19: Diagram of axis into show "histogen" regions. A, longitudinal section B transverse section.

Where the initials are in a cluster or mound, the outer ones, if their divisions are strictly anticlinal, form a definite tunica, and the inner ones, with divisions in more than one plane, form a corpus (Figs. 3.18 D, 3.20 D 3.21 C, D). Under these conditions tunica and corpus have completely independent origins and are fairly distinct. Between these extremes lie, many intermediate conditions in which distinction of tunica and corpus is weak or doubtful.

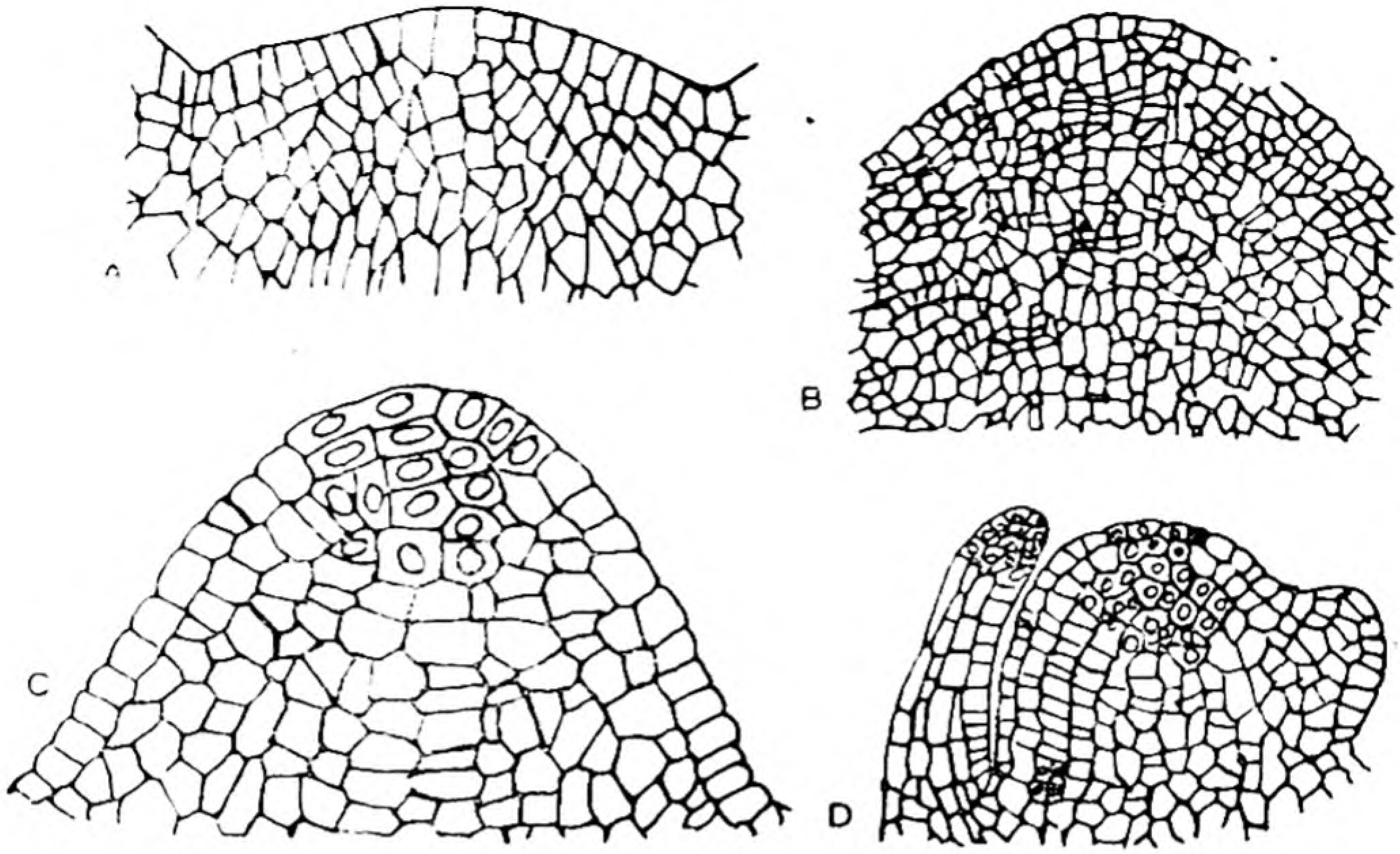

FIG. 3.20: Structure of stem apices as seen in longitudinal section. A, Lycopodium Selago, apex built up by superficial initials which divide both anticlinally and periclinally. B, Zamia umbrosa, apex built up by central group of superficial initials which divide both anticlinally and periclinally. C, Sequoia sempervisons, apical initials divide both anticlinally and periclinally; subapical initials divide in all planes (initials indicated by outlined nuclei). D, Sinocalamus Beecheyada, tunica two-layered, its initials in two layers, with divisions, with rare exceptions, anticlinal; initials of corpus many, in a mass below tunica intials, their divisions in all planes (initials of apex and of leaf apices and bud indicated by outlined nuclei). The heavier lines in C and D indicate not thicker cell walls but separate zones, limiting in C, a surface layer, the dermatogen, and a central core that forms the pith; in D, the two tunica layers, and the central part of the corpus, formed by periclinal divisions of the lowest corpus initials. (A, after Haertel; B, after Johnson; C, after Cross; D, after Hsu, modified.)

Below the initials or below the direct derivatives of these cells (the two groups constituting a promeristem), elaboration of cells in size, form, and arrangement initiates tissue specialization and the building of the framework of the primary body. The various regions, or stages, merge on their margins and overlap longitudinally. Major interruptions in the progressively more advanced stages back from the apex are brought in by the appearance of nodal areas and lateral structures.

Types of Stem Apices

In vascular plants, zonation in stem-apex differentiation follows more or less definite patterns that appear to be characteristic of the major groups. These patterns show increasing complexity from the lower to the higher groups and seem to

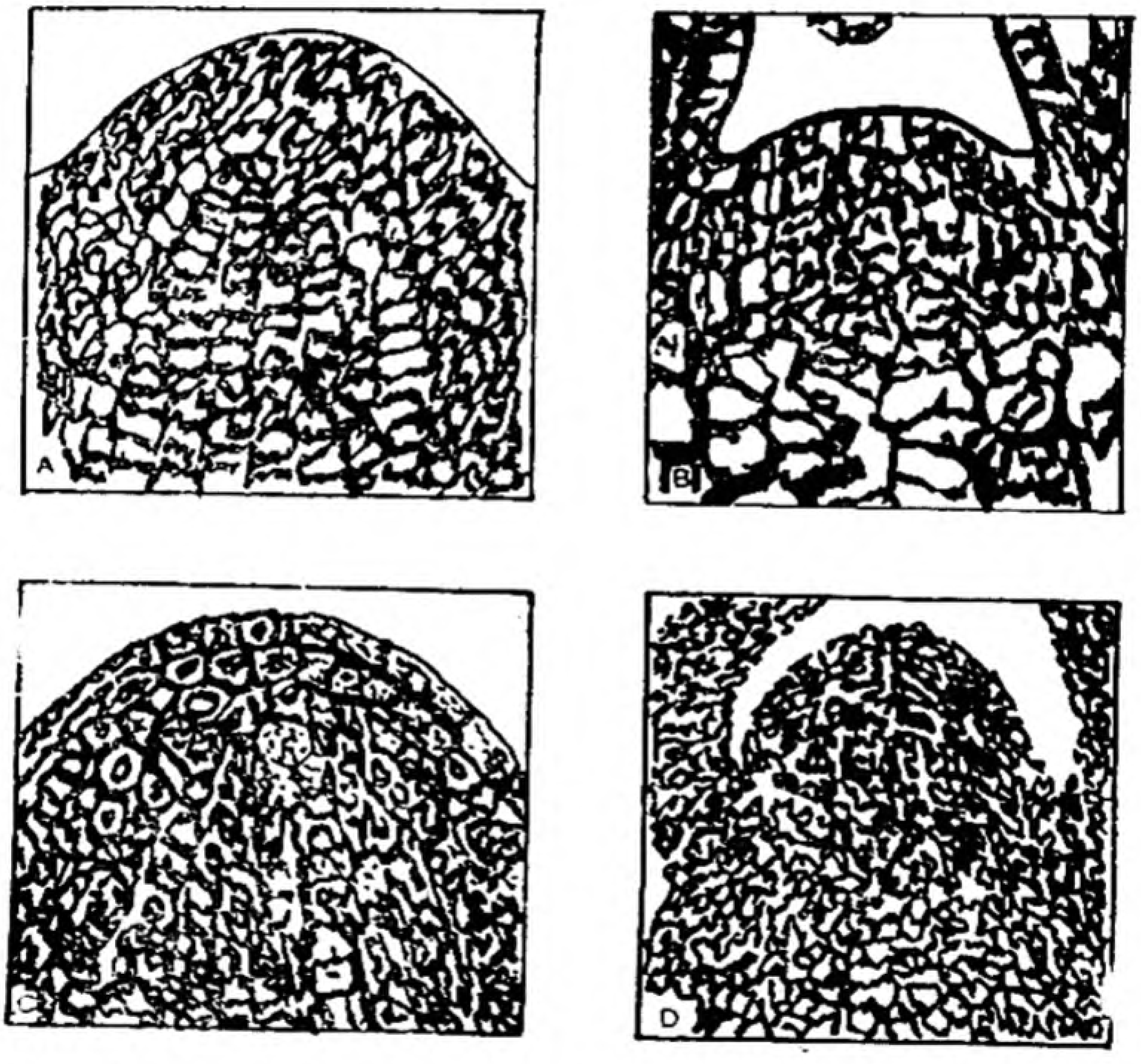

FIG. 3.21: Structure of stem apices as seen in longitudinal section. A, Abies concolor, initials of the surface layer divide both anticlinally and periclinally, subapical initials in all planes; B, Sequoia sempervirens, initials of the surface layer divide chiefly anticlinally, subapical initials divide in all planes; C, Vinca rosea, tunica of three layers and corpus present, initials of tunica dividing only anticlinally, those of the corpus dividing in all planes; D, Phlox Drummondii, floral apex. (A, after Korody; B, after Cross; C, after Cross and Johnson; D, after Miller and Wetmore).

represent a series in specialization from simplicity to complexity. In lower groups the initials form a uniseriate surface layer, and there is no evidence of differentiation into tunica and corpus; in the highest groups the initials form a two-storied cluster of which the superficial members build a tunica—which, in its greatest specialization, is probably uniseriate—and the inner members form the corpus. The majority of seed plants are intermediate in apex structure between these types.

The primitive type of stem apex. Among the pteridophytes, *Lycopodium*, *Isoetes*, and some species of *Selaginella*, and among primitive gymnosperms, the cycads, have simple apices with surface initials and no distinction of tunica and corpus.

Lycopodium (Fig. 3.20 A) serves as an example of this primitive type. The initiating layer in a weakly defined, uniseriate surface area which divides freely both anticlinally and periclinally. No definable initials can be distinguished; all the cells of the layer are morphologically alike. The anticlinal divisions increase the area of the surface layer; the periclinal division forms on inner core.

The stem apex with weak tunica-corpus segregation. Beginnings in distinction of tunica and corpus are perhaps present in some of the lower conifers. In the Pinaceae (*Abies*, *Pinus*), the initials form a terminal uniseriate group. From these, by both periclinal and anticlinal divisions, a central core and an enveloping uniseriate layer are formed (Figs. 3.18 C 3.21)). The latter suggests a tunica in appearance; but it has frequent periclinal divisions even on the flanks of the apex, and there is no clear line of separation between the tissues formed by the two regions.

Some higher conifers have a somewhat more specialized apex (Figs. 3.20 C3.21 B). As in the Pinaceae, the initials are a small group of surface cells in one tier, with divisions both anticlinal and periclinal. These divisions form, respectively, a dermatogen-like layer and a central mass, but in the outer layer the periclinal divisions are rare or absent, except close to the apex.

The part played by the outer layer in building the body of the stem is restricted to the cells at the very apex. The outer layer early becomes tunica-like in its restriction of planes of division.

Of the conifer genera thus far critically studied, only *Crytomeria* and *Taxodium* (apices of permanent shoots) have a "dermatogen" in which there are no, or almost no, periclinal divisions. The apices of conifers have the structural appearance of tunica-corpus segregation but in those so far studied there is only one tier of initials and therefore no independent meristematic regions

The conifers show much variety in apex structure. Even within a species (*Taxodium distichum*, *Sequria sempervirens*) there are marked differences, correlated in part with morphology of the stem concerned, vigour of growth, and other conditions.

The stem apex with definite tunica and corpus. In the angiosperms the segregation of apical-meristem zones in usually more definite than in lower groups; there are two sets of initials, one above the other, which give rise to tunica and corpus that are in large measure more wholly independent (Figs. 3.18 D, 3.20 D 3.21 C). The tunica has no only rare periclinal divisions and ranges in thickness from several layers to one, with two or three layers probably most frequent. The large numbers of tunica layers occur more frequently in the dicotyledons. A single-layered tunica such as that of grasses (*Avena*, *Triticum*) probably represents the most specialized condition, but even in this, occasional periclinal divisions may occure, as in *Zea*. The corpus varies from a large complex type to a slender, simple type. The number of layer in the tunica may vary even in an individual plant. The limits of the two groups of initials and of the tunica and corpus is often definite but may be difficult or imposisble to determine and in some genera (Cactaceae) the apex shows no distinction of tunica and corpus, resembling the apex of the primitive gymnosperms. *Sinocalamus Beecheyana* (a bamboo) (Fig. 3.20 D) and *Vinca rosea* (Fig. 3.21 C) serve as examples. In the former the tunica is two-layered (sometimes one or three layered); in the latter it is four-layered.

Discussion, tunica-corpus theory. The tunica-corpus theory has served well in the establishment of an understanding of the complex, diverse, any varying meristematic patterns of the stem tips of seeds plants. Providing a basis and a method, it has stimulated intensive study, and detailed information has been obtained for a considerable number of plants. The position, number, and behaviour of the initiating cells in seed-plant stems is now in some measure known, and early stages in the development of the primary body of the shoot are much better understood. Although, as with the histogens of the histogen theory, a distinction of tunica and corpus has little or no morphological significance, it is of topographical value in studies of detailed development. Caution is necessary lest morphological interpretations accompany the use of the theory: the boundaries of tunica and corpus are often obscure; where the limits are fairly distinct, the two regions may be inconstant in structure and function, varying with seasonal conditions, vigour of growth, age of plant, position on plant, and morphology of the stem tip concerned. For example, in some plants the innermost tunica layer of a primary branch becomes an outermost part of the corpus on secondary branches. The number of tunica layers varies even in apices of the same type at the same time; it is commonly less on the less vigourous and the lateral branches than on strong, leading stems. The differences found, even on the same plant, in the thickness ann distinctness of the tunica at different times and under different morphological conditions—as in main and lateral branches, deciduous and persistent twigs, indeterminate veegtative tips and the determinate apices of flowers and thorns —are related directly to growth status and to the morphological nature of the developing organ. A flexible basis is necessary for all descriptions of meristems. Tunica and corpus should be recognized as dynamic and fluid, not functionally or morphologically distinct or constant regions.

The lateral organs of the stem—leaves, branches and floral organs—arise near the apex and studies of tunica and corpus have added greatly to a knowledge of the origin and early development of these organs.

The Floral Apex

The structure of the floral apex (Fig. 3.21) differs in no fundamental way from that of the vegetative stems. Such differences as can be distinguished are those inherent in the determinate nature of the axis, the telescoping of internodes, and the crowding of appendages.

It has been claimed that the floral apex differs markedly from the vagetative apex; that apical initials build up the "central core" in the vagetative axis, but that both apical and flanking tissues build up the floral apex; that the tunica of the floral apex has no definable initiating zone and that periclinal divisions occur at any depth within it. Such differences may occure, varying greatly in degree with the form and structure of the flower concerned, but they are not morphological differences. The are associated directly with the function of an apex that is developing into a flower—elongation ceases; the apical initials of the earlier vegetative growth lose their identity, and growth activity is restricted to the peripheral region where many appendages are arising at compacted nodal levels; the numerous periclinal divisions at all depths are associated with the broadening of the receptacle and with the origin of the floral appendages which cover it. Distinction of outer and inner zones varies in the floral apex as it does in the vegetative apex. The number of layers in the tunica of the floral apex is more in some plants, less in other, than in the vegetative apex. In ontogency a floral apex develops by the gradual or abrupt transformation of a vegetative apex.

The Root Apex

The apical meristematic region of the root, lacking developing appendages and segregation into nodal and internodal regions, is simpler in gross structure than that of the stem but is complicated at the tip by the *root cap* and different methods of formation of this structure. The root cap is a terminal portion of the root tip, more or less definitely set off from the tissues beneath, covering and protecting the initiating apex of the root proper. It is formed by the same initials as those that build the root proper in all types of root tip except those of the monocotyledons where it has independent origin. Its formation is necessarily discussed with that of the root apex.

The apical meristem is short compared with that of the stem; specialization into zones is concentrated, with the early stages lying within the terminal millimeter beneath the cap. Growth proceeds in cap and root proper in opposite directions, with sequence in development towards the tip in the cap and away from the tip in the root itself. Ih number, the initials range from one to many (Fig 3.21). Where the initials are more than one, ther are arranged in one to four fairly distinct, uniseriate groups (Fig. 3.22B, C, D). In each group there are one to several initials. Since the initials are the central members of uniseriate layers and can be distinguished from their recent derivatives only by position and restriction in division to anticlinal planes, the number can often be determined only approximately. Furthermore, the number of initials in a group seems to vary with diameter and rapidity of growth of the root. In slender roots it may be reduced to one—as in some grasses—but the zonation remains clear. Lines of zonal segregation are most obsecure in root tips of large diameter. Where there is more than one group, the groups lie adjacent to one another on the longitudinal axis of the root (Fig. 3.21).

Each of these groups quickly develops one or more growth zones which are usually more clearly marked than are similar zones in the stem apex In many plants these zones appear to represent "the histogens", and interpretations of the root apex have long continued under the histogen theory. Although the terms dermatogen, periblem, and plerome are no longer in general use in descriptions of stem ontogeny, they have been continued for convenience to indicate general zones in studies of root development. A fourth histogen, the *calyptrogen*, is added where the cap has an independent origin.

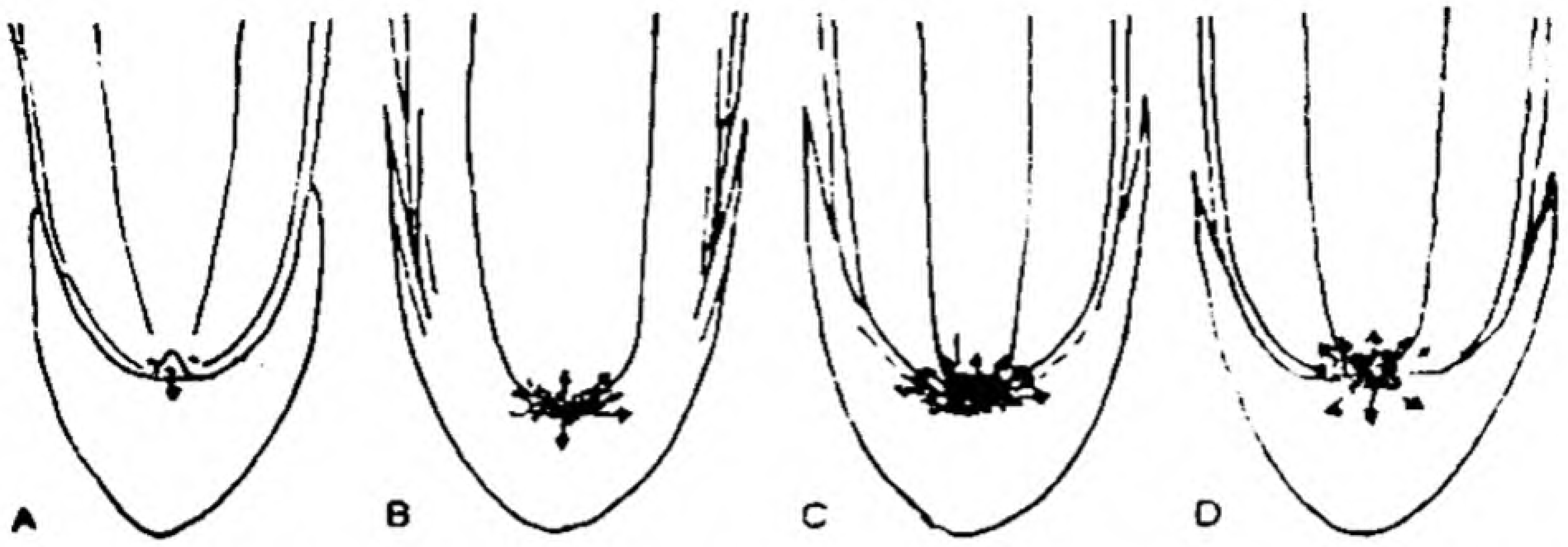

FIG. 3.22: Diagrams of root-apex types. A, initial solitary, cap distinct, but not independent in origin; B, initials in two groups, cap not structurally distinct, formed by same initials as periblem and dermatogen: C, initials in three groups, cap not distinct, formed by same instials as dermatogen; D, initials in three groups, cap distinct in structure and independent in origin.

In published descriptions there is much confusion as to method of origin of the zones and no agreement as to the number of types of development. The distinctness of the zones varies, and forms intermediate between the types have been described. There seem, however, to be basic patterns for the major plant groups. This pattern is determined by the number of initials, the number of groups of these intitials, the zones formed by each group, the morphological nature of the cap, and the degree of independence of the cap.

The vascular cryptogams that have a soliditary apical cell in the stem—the horsetails, most of the ferns, some species of *selaginella*—have a similar solitary apical cell in the root (Fig. 3.22A), This one cell forms the entire root and the cap which is usually sharply distict structurally. The origin of the cap from the lagre apical cell is clear.

In many gymnosperms there are two groups of initials (Fig. 3.21). The inner forms the plerome; the outer forms the periblem and the cap. No line can be drawn between these two regions; the cap appears as a distal poliferation of the periblem.

4

Cell Morphogenesis

The plant cell is typically delimited by its wall. There are potentially two kinds of cell morphogenesis for such cells because new wall surface can be generated either by formation of a new cross-wall or by expansion involving the pre-existing wall. In the first case, new cells of varying proportions and reduced volume are relatively instantaneously produced by the completion of a cross-partition after mitosis. In the other case, which will be of primary concern here, cell proportions are gradually changed (or magnified) by an enlargement of cell volume and surface already present. This generation of new volume by the yielding of the cell wall to the stresses within it is termed growth by the present reviewer.

Each mode generating cell shape (partitioning versus expanding a given volume) is readily recognized when it occurs alone. For example, in the regeneration of vascular strands, new phloem cells are "cut out" of parenchyma cells solely by cell division. No deformation of wall structure appears to be involved; wall synthesis is obviously required. On the other hand, the elongation of a root hair or the inflation of a giant algal cell such as the internode of *Nitella* has its immediate basis in the expansion of pre-existing surface. This is shown by the fact that marks put on the pre-existing surface separate from one another. That such movement ultimately requires synthesis is unquestioned. The overt morphogenetic phenomenon is, however, a rehelogical one and must be dissected into its various physical components before meaningful ties to synthesis and other factor, such as wall stresses, can be made.

Because cell division, viewed here as a partitioning process, cannot contribute significant new volume to a developing system, its involvement in organ morphogenesis must be indirect. A particular division pattern could promote, through altered cell physiology and cell physics, a particular pattern of deformation of cell volume which would generate on organ or complex form form. The development of the red alga *Callithamnion* provides a striking example. Each cell in the simple uniseriate main axis may expand to produce a single outgrowth or branch. Successive cells alternate in making the outgrowth on the left versus the right side to give the flat thallus a feather-like appearance (Fig. 4.1). The swellings or outwrowth arise on the longest part of the cylindrical surface of the axial cells. The alternating (left versus right) location of this long side in successive cells has its origin in an alternating oblique mitosis of the apical cell. Recently made apical cells from only transverse cross-walls, so there is no long side in the derived cell; in this case no outgrowths arise. In this plant the geometry of wall deformation reflects a previous pattern of mitosis. In organs already initiated, however, there is evidence that cell division relates to histological pattern (as it must in any situation) but is not involved in the course of organ morphogenesis. The normally occurring transverse cross-walls are eliminated when developing leaves of wheat seed lings have received gamma-irradiation. The geometrical course of leaf enlargement is normal despite the fact that leaf histological pattern is altered by the presence of abnormally long cells. In this case, the enlargement of cells by distortion (stretching) of the side walls is a sufficient mechanism for the morphogenesis of the organ.

The mode of cell morphogenesis based on cell expansion is

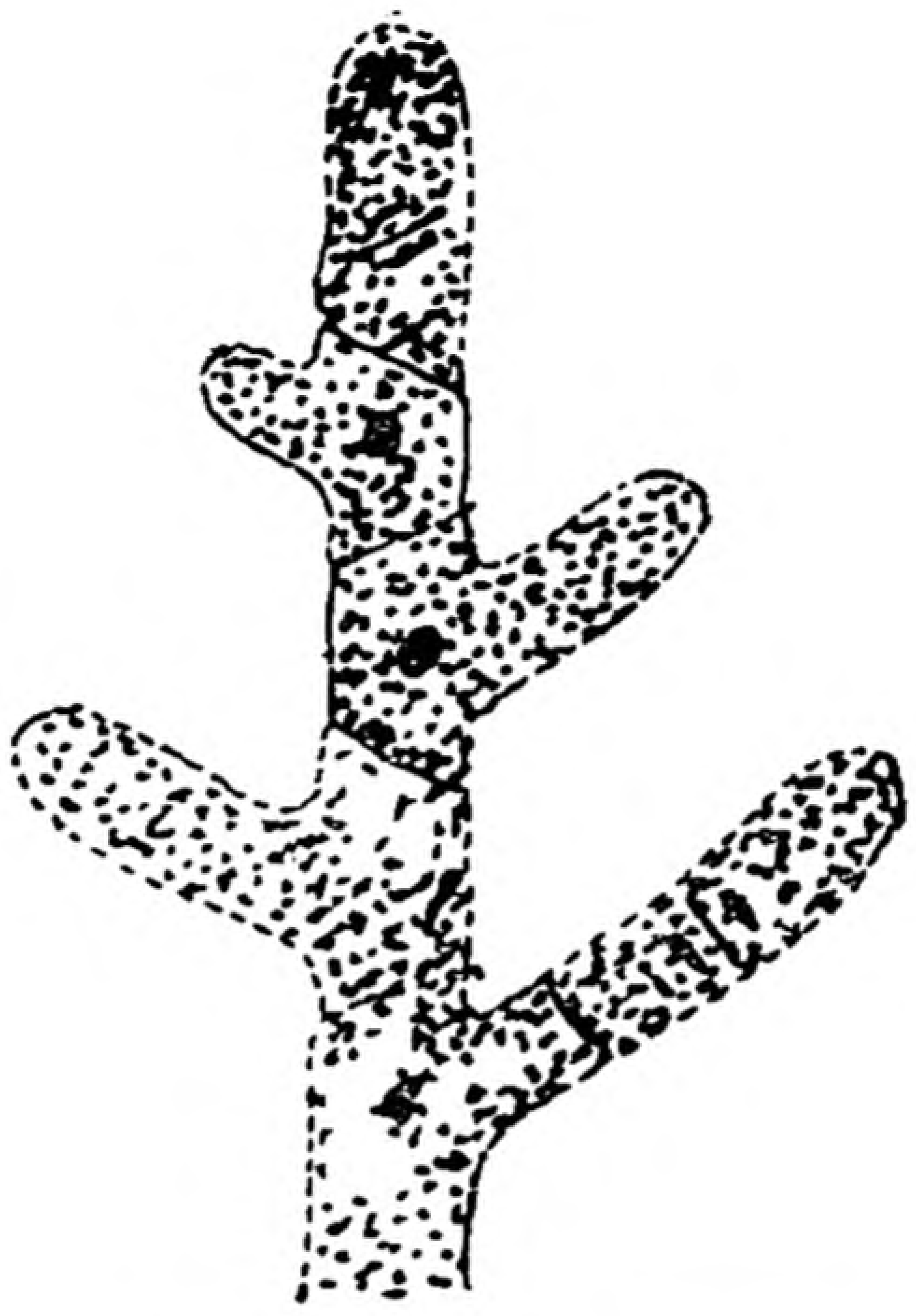

FIG. 4.1: Shoot tip of the red alga Callithamnion. Alternating oblique mitoses in the tip cell produce calls with one long side. This side produces the lateral. Hence the pattern of surface expansion is coupled to previous mitotic events.

thus generally significant in plant development and will form the basis of this review. For simplicity it will be examined when it occurs unaccompanied by cell division. We will start to examine the links between cell deformation and its ultimate basis in structure, metabolism, and genetics by considering the immediate cause of change of shape.

The indirect origin of from cell structure. A few model examples will show that it is essential to resolve the gross change in shape of a cell into a pattern of local deformations of cell surface before proceeding to deeper questions of mechanism. In the models the shape is always a figure of revolution, and the local deformation behaviour is always the same around a given circumference. In this way a 3-dimensional structure can be analysed in terms of only 2 variables.

height and radius. In Figure 4.2, a model cylindrical cell becomes transformed into a trumpet shape.

The pattern of expansion, as resolved into the behaviour of small subdivisions of surface, cannot be deduced from the change in cell outline; it must be derived from analysis of material points (wall ornamentation, applied marks, etc.). In the diagram the pattern of local surface change is revealed by the deformation of circles attached to the surface. In *a* the morphogenesis is achieved by a gradient in the rate of surface expansion, the circles remaining circles as they enlarge at different rates. This mode of shape change, a gradient in the rate of isotropic (equal in all directions) surface expansion, is generally the first one to come to mind. Such a pathway for shape change has its obvious potential mechanism in gradients of substances that promote or inhibit expansion rate. As sequence *b* shows, however, the above mode of expansion is not the only one that will produce the same shape change—by the same route of intermediate cell outlines. In *b* we observe a gradient in the direction of surface expansion, the transverse component being strong at top the and weak at the bottom, and the longitudinal component behaving in the opposite way. There is no gradient in the rate of area expansion in this example. Clearly, many intermediate mixtures of these two extreme pathways could being about the same change in form.

It is important to note that the second pathway involves variation in direction of maximal expansion. Change in shape brought on by oriented local expansion generally has no obvious immediate explanation in terms of diffusing agents (inhibitors, stimulators, or gene products in any simple sense), but rather must be based on oriented features of the cell such as stress anisotropy or organized wall texture. This oriented stretching or expension may align cytoplasmic structures such as chloroplasts and microtubules and thus may have effects in addition to the obvious one relating to the shape of the cell. In studying a change in form, it is therefore fruitful on several grounds to first resolve the observed change in cell outline into the behaviour of small subdivisions of elements of the cell surface. It is this behaviour, not cell form, which can be expected to relate to the details of cell structure and cell physiology.

That local behaviour cannot be inferred from the character of cell outline (unless it has natural makings) is also evident in tipgrowing cells where expansion is confined to a roughly hemispherical growth zone (hyphae, pollen tubes, root hairs). Such zones continually produce a cylinder at their base. Each point between the very tip and the base of the dome undergoes an apparent migration away from the tip (growth above any such point appears to move it downward). The character of surface distortion of a small region on the hemispherical dome

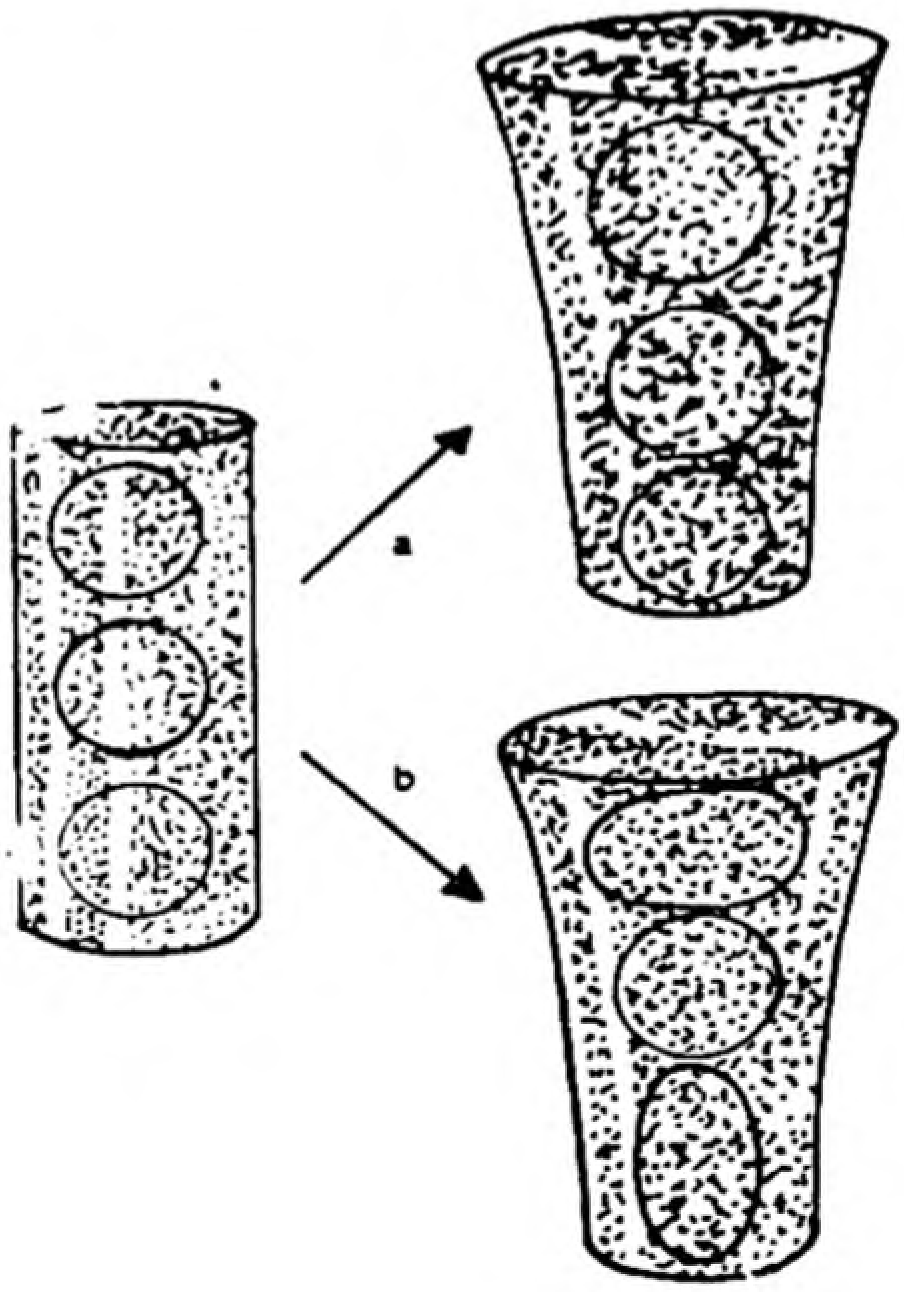

FIG. 4.2: Two extreme pathways for the deformation of a cylinder to a flaring trumpet shape. Local regions of surface are delimited by circles. In *a* shape changs occurs in response to a gradient in the rate of expansion of area. The circles enlarge at varying rates but remain circles. In *b* there is no gradient in rate of expansion (final circle and the elipses have the same area) but there is a gradient in the direction of expansion. (After Green.)

could take many forms, all indistinguisable unless marks on the surface are followed. A small region may be continually enlarged without distortion (Fig. 4.3A), may be stretched out along the cell axis (Fig. 4.3B), or may even be primarily stretched laterally along the cell circumference as in Figure 4.3C). The same possibilities appear present in the initiation of a new axis of growth either in a germinating structure or in the branching of a cylindrical cell.

In brief, a mechanism of cell morphogenesis must account for a succession of forms, not form *per se*. The geometrical course of development of a cell can be viewed as the integral, through space and time, of the behaviour of small districts of the cell surface. Such behaviour, which is described in terms of both the rate and directionality of expansion, will in turn have its origin in the distribution of structural and physiological parameters throughout the cell. Useful reviews of cell wall structure are written by many scientists.

Scope of the rewiew. To a somewhat unusual degree, discussion will center around the morphogenesis of cells with exposed surfaces (plant hairs, filamentous and unicellular lower plants). The stresses within the wall are generated primarily by the cell itself rather than by adjacent tissues.

Cells in tissues are potentially subject to tissue tensions whose magnitude and role are hard to evaluate. For example, the stellate pith cells of *Juncus* have radiating arms connected to other cells via arm tips. If is not clear whether the cells are "pulled out" into this configuaration by growth of the epidermis or whether they "push", end-to-end, to increase the diameter of the epidermis. The first alternative is favoured by the observation that rapid epidermal growth in stamen filaments can tear the inner cells apart. A pushing role is favoured by the finding of a transverse arrangement of cell wall microfibrils typical of actively elongating cells. Uncertainty as to the origin of the physical forces deforming the cell delays further analysis.

A strong possibility of tissue effects is found in the development of the palisade layer of leaves where a nearly spherical cell elongrates into a cylinder. Slow growth in area of the two surfaces of the leaf would inhibit side-wise expansion of these cells and promote, if not cause, the directed character of their expansion. Excised palisade cells do not elongate but tend to swell in the middle. They would favour "imposed" derectionality of the previous elongation. On the other hand, the elongating cells do have the cell wall fine structure normally associated with independent elongation.

A further variable assocated with tissue cells is a more complex wall structure. Thick outer walls of epidermal cells differ in structure from walls in adjacent parenchyma. Parenchyma cells themselves contain longitudinal "ribs" of aligned micofibrils. It has been suggested that these ribs are the result of synthesis of cellulose inside the wall. However, there is an argument a that these microfibrils reach their unusual position by a sliding process brought on by tissue tensions. The ribs are missing in the exposed arms of the *Juncus* stellate pith cell. Further scientists found little or no synthesis of cellulose in the interior of the rapid epidermal wall this may be general. These questions are reviewed by others.

The Elongating Cylindrical Surface

Many cylindrical cells shift their proportions in favour of increased length by virtue of highly directed (anisotropic) surface expansion occurring along the entire length of the cell. This has been demonstrated with marking studies for the epidermis of *Avena* and for the in ternode of *Nitella*. An important study of the number and spacing of primary pits in deeper plant tissues established this pattern of expansion for cortical cells, in two aquatic angiao sperms (at least in the later stages of growth). It may well characterized dividing cells.

The directed aspects of expansion can be characterized in some cases by a single constant: the ratio of the relative growth rates, each expressed as (fraction/time), in the axial and transverse directions. A constant rate found in both *Nitella* and *Hydrodictyon* correlates with a constancy in the wall's transverse microfibrillar alignment as measured by the quotient the tradation in polarized light/optical thickness). The constant wall texture presumably gives the wall a constant anisotropic yielding quality. This, when integrated over space (the cell surface) and time, gives the cell its characteristic shift in proportions during development.

The typical wall structure for such elongating surfaces has, in parenchyma and certain algal cell walls, in over-all transverse (hoop-like) arrangement of birefringent collulose micofibrils. It has long been known that the stress pattern in a cylindirical wall favours increase in girth. Therefore the transverse arrangement of microfibrils must so strongly reinforce the wall in that direction that most of the expansion occurs in the axial direction. This has received direct support in a study by several scientists of the mechanical properties of isolated longitudinal and transverse wall strips of *Nitella*. Microfibrils appear to govern the directionality of growth and thus the shape of the cell.

Since the classsical work of the late Professor Roelofsen and collaborators it has been recognized that the over-all transverse microfibrillar arrangement is actually a steady-state condition—a balance between continual deposition of transverse microfibrillar material at or near the wall inner surface and the realigning action of cell extension, "muld-net growth." Once a given group of microfibrils has been deposited, it suffers both thinning and eventual passive realignment into the longitudinal direction. There is a gradient in microfibrillar orientation from transverse to longitudital as the growing wall is examined from the inner surface to the outer. The transition to longitudinal orientation occurs near the wall outer surfaces; so the over all orientation is transverse. The microfibrils thus rotate in a plane tangent to the cell surface; they apparently do not rotate around their own long axis because one crystallographic plane is typically found parallel to the cell surface.

That the observed microfibrillar pattern on the wall inner surface is adequate to maintain the less well ordered over-all arrangement in *Nitella* cell walls has been shown by scientists. They examined the possible frictional relation between the microfibrils and the matrix in light of the directed growth of cell the and physical properties of isolated strips of wall. The relation of Bingham flow (linear increase in rate of flow above a threshold value of stress) gave the best fit. Their analysis led to the prediction that at low turgor a cell should grow broader in proportions than at high turgor. This is, cell shape would be a function of the wall stress in excess of the yield stress, but not of the viscosity of the matrix.

A major exception to the correlation between elongating cylindrical surfaces and transverse wall texture is faund in the algal *Chaetomorpha*. The cell wall here has two kinds of lamellae which alternate within the wall. One kind has well-ordered microfibrils living in a slow spiral and the other has a steep spiral. In some species, a third kind of lamella with microfibrils at an intermediate angle can be detected. This kind of wall structure is called "crossed fibrillar" and is common in certain groups of algae, including the ovoid cells of *Valonia*. It may explain the twisting growth in *Chaetomorpha*.

While the most widespread crystalline microfibrillar component is cellulose, in fungi chitin (and possibly other aminopolysaccharides) contributes to wall structure. Mannan and xylan are the major structural elements of walls in certain algal groups and in endosperm. In certain fungal walls, X-ray diffraction shows that crystallinity appears only after chemical treatment. In cellulose walls, microfibrillar alignment correlates well with the alignment of microtubules in the adjacent cytoplasm.

Loss of the normal directionality in elongating cylindrical surfaces; loss of microtubules? There are several instances of elongating cylindrical surfaces—growing along their length—changing their expansion pattern so as to produce a spherical cell. In *Hydrodictyon africanum* this occurs in normal development and is accompanied by a shift in wall structure as determined with polarized light, from a predominantly transverse arrangement to a configuration with no over-all orientation. In *Nitella* a similar loss of the capacity for axially directed expansion and the same shift in wall texture is brought on by growth in colchicine a drug which breaks up microtubules and disorganizes secondary wall synthesis. The drug had long been known to produce gross swellings and relatively isodiametric cells in plants. That change in texture was brought on directly by the drug (*i.e.*, it was not a consequence of shape change) has been shown by the drug-induced production of randomly oriented microfibrils on the inner wall surface of cells mechanically restrained from changing form. This implicated structures of spindle-fibre character (microtubules) in the control of oriented cellulose synthesis.

Many chemically diverse compounds produce a sphering effect. Only rarely has an investigator followed the shift in expansion direction, the ultrastructure, and the chemical composition in one system. Benzimidazole, in the presence of auxin, causes cells of the pea epicotyl to elongate less than normal and to increase in diameter considerably more than normal. Is was found that wall chemistry was not appreciably abnormal, but wall structure showed an accumulation of many new longitudinal microfibrils in the region of the longitudinal "ribs" typical of dense tissue parenchyma. This kind of reinforcement could explain the shift in direcrion of growth provided the longitudinal cellulose bears stress adequately. It was concluded that the drug induced deposition of longitudinally oriented cellulose in the interior of the wall. Since deposition in the wall's interior appears not no occur when directly tested in the normal development of *Avena* coleoptile cells, other explanations may apply for the pea epicotyl's response. By analogy with the above-mentioned cases in exposed cells, the low negative retardation between ribs—as seen in polarized light—could reflect disorientation of cellulose. This might cause the unusual increase in girth.

There is little structural information on the "sphering up" of cylindrical cells brought on by other reagents, High concentrations of auxin apparently promote increase in diameter at the expense of length in *Helianthus* hypocoty tissue. There is no strong reason to suspect an action on microtubules, although in tissue culture auxin does appear to suppress the formation of microtubules in carrot cells. Generally, auxin is believed to "loosen" the matrix components of the wall, so it is possible that excessive loosening diminishes the interaction between microfibrils so that, even though their initial alignment is nearly normal, the effective anisotropy of strength of the wall is lowered and this results in sphering. The herbicide trifluraliu, a mitotic poison, causes swollen cells in higher plants. In *Nitella* it produces spherical cells from young cylindrical internodes. The surface behaviour during the transition has not been followed, but the wall texture does shift from predominantly transverse to random (all microfibrils lying in the plane of the wall).

Ethylene, whose production may be a consequence of auxin action, produces swollen cells; it also promotes the initiation of root hairs. Malformin, isolated from fungi, causes cell swelling, probably by the induction of ethylene formation.

Because heavy water stabilizes and enhances the birefirngence of the mitotic spindle, it might be expected that it would counteract the effects of reagents like colchicine and, if simply included in a normal growth medium, it might be expected to increase the directionality of growth. The reverse effect, however, a swelling of the organ, appears typical. The forces aligning microtubules in the cell cortex and the spindle may be different.

The surface behaviour converting elongation to spherical inflation has apparently not been studied in the above cases. This behaviour probably presents one of the simpler entries into experimental morphogenesis for structural-physiological investigation.

Control of oriented cellulose synthesis. The few studies relating mitotic poisons (which disrupt spindle fibres) to the sphering up of cells implicate microtubules in cellulose orientation despite the fact that the cellulose and microtubules lie on opposite sides of the cell membrane. This situation need not preclude an orienting role for microtubules because the geometrical details of microfibril synthesis and the attendant membrane behaviour, with or without specific orientation, are unknown in eukaryotic plants

Certain particulate structures have been proposed to govern cellulose synthesis and orientation. In freeze-etch preparations, particles are seen on the outer surface of the membrane and in some cases in the cell wall itself. Fibrous extensions from the particles appear to be microfibrils, although similar extensions are occasionally seen in plastid particles. The cell types where these particles were first described (yeast, *Chlorella*, and blue-green algae) do not have well-oriented microfibrillar patterns, so a correlation between microfibrillar orientation and particle alignment (comparable to that between microtubules and cellulose) could not be made. Recently, scientists have seen aligned particles on the outer surface of the plasmalemma in root cells with oriented cellulose. The strands of particles appeared to vary more in orientation and to be very sparse compared to microtubules or microfibrils. Fields showing both abundant parallel microfibrils and membrane or abundant microtubules and membrane showed no obvious corresponding aligned pattern of particles on the membranes. The particles were also noted on fractured starch grains.

A clear correlation with larger (cytoplasmic elements is seen, however, in shadow-cast replicas of *Chaetomorpha* walls from which the cytoplasm has been removed by plasmolysis. Here linear aggregates of cytoplasm (particles or clumps) are seen parallel to the prospective orientation of the next layer of aligned cellulose. It is conceivable, however, that plasmolysis removed a thin wall layer and that the particles reflect rather than anticipate wall orientation.

A diversity of roles has been proposed for the particles seen in or near cell walls. They could serve as initiation centers for microfibrils, as cross-linking *Haftpunkte* between microfibrils or as elements of a three-dimensional lattice through which microfibrillar elongation would proceed only in preferred directions. In the last view, the particles would have the property of catalyzing the addition of residues to microfibrils along any of three lines through the particle. In a tight lattice this would permit synthesis to proceed without movement of microfibril or enzyme, but would require truly remarkable catalytic properties for the 500 A particle.

The particles seen in freeze-etch material have been proposed by botanist as the form in which a cell wall protein "extensin", is present in walls. The hydroxyproline rich material is thought to be specific to cell walls and involved in their growth. This view has been challenged recently by several scientists, who found that radioactive proline is not found to be concentrated in cell walls of carrot.

In brief, a role for particles in wall synthesis cannot be excluded, but a strong particle alignment with a relation to known microfibrillar orientation is seen only for large particles in *Chaetomorpha*. In many walls with aligned microfibrillar texture, there is a parallel arrangement of microtubules and cellulose microfibrils. This suggests a causal role for the former in oriented synthesis, and thereby in the succession of cylindrical forms assumed in elongation.

De novo origin of elongating cylindrical cell walls. The wall-less and motile swarmer cells of *Hydrodictyon* assemble into a net pattern on the inner wall surface of the parent cell. The cells elongate as cylinders from points of contact and appear, in polarized light, to have walls with transverse texture. It is not known if there is a transitory first-formed wall with random microfibrils as found in germlings of *Chaetomorpha* and *Cladophora*. Wall texture in *Hydrodictyon* may have its origin in some structural feature of the swarmer; directionality based on stresses generated by cell-versus-cell "pushing" seems unlikely because the net could bend before appreciable stresses could be produced.

Origin from apical cells. The surface of the *Nitella* internode comes ultimately from that of a single apical cell. Assuming that surface elongation is based on transverse microfibril deposition and that this in turn is somehow controlled by a transverse arrangement of microtubules, there are two major questions:

(a) How do the microtubules retain a transverse position in the face of longitudinal surface extension; and

(b) how do they initially achieve this position? Only highly tentative answers can be given.

Conceivably, retention of orientation could be based on a hoop-like arrangement of the microtubules. As long as the microtubules, singly on in aggregate, ringed the cell vacuole, they could not be strain-oriented into the longitudinal direction without breaking or extending, as chords through the cell interior. This method of maintaining transverse alignment in spite of longitudinal strain was suggested long ago for microfibrils.

The elongating surface of an internode is derived from the surface of the apical cell. This cell has a random pattern of microfibrils and presumably a random arrangement of microtubules in the cell cortex. Marking experiments show a predominating transverse stretch at the base of this cell; this could be the process which first gives the microtubules their preferred orientation. Once transversely aligned, they would he expected to be associated with transverse microfibrils whose presence would redirect expansion into its definitive longitudinal character. Microtubular details over these stages are unknown, but transversely oriented cellulose can be directed *in vivo* shortly after the temporarly circumferential stretch. This stretch could in turn have its origin in a gradient of wall thickness or extensibility in the apical cell wall.

This view that oriented strains can establish the normal transverse microfibril synthesis is bolstered by the observation that a new (branch) axis of growth —with wall structure appropriate to that axis—can be mechanically induced in *Nitella* internodes.

Consequences of oriented expension of cylindrical surfaces. The directed stretching of cell surfaces can influence the subjacent cytoplasm. In *Nitella* internodes a slightly oblique (nealy transverse) wall texture is capable of explaining the twisting aspect of elongation characteristic of that cell. The total amount of twist measured on the wall surface corresponds to that shown in the parallel chloroplast files, so the surface growth pattern appears to strain-align the chloroplast files. Parallel to the plastid files, and just interior to them, microfilaments can be seen in the electron microscope. They are apparently at the site of the motive force for the protoplasmic stream. Therefore the strain pattern of the wall, acting to align the plastid layer, would ultimately establish the helical streaming pattern.

Chloroplasts in a young cell which has been mechanically inhibited from expanding lose both the directed character of their expansion and their capacity to divide. Thus the oriented behaviour of certain organelles can be shown to be ultimately dependent on the oriented behaviour of the cell wall. The apparent helical alignment of phytochrome in *Mougcotia* could be based on previous helical growth of cell surface, possibly extending this generalization to the molecular level.

In cells which are both elongating and dividing, cross-walls are usually at right angles to the direction of maximum expansion. This correlation is valid for both long and short cells in rib meristems, the cambium, ete., so some connection between these two directed aspects of development could be sought.

The exceptional case of the normal division of the guard mother cell of stomata can be brought into conformity with this generalization after chemical treatment. The effective reagents after the timing of preceding mitosis, thereby altering subsequent cell shapes and strain patterns. By exaggerating longitudinal extension, gibberellic acid appears occasionally to reorient a division plane in *Avena* stomate formation. The site of junction of a future cross wall and the side wall is marked by microtubules before division.

In summary, the directed expansion of cylindrical and surfaces appears based on continuous oriented wall synthesis which is related to transversely aligned microtubules. The origin and maintenance of this alignment is far from certain. A correlation between a transitory circumferential stretch and the origin of transverse wall texture can be seen, however, in early developmental stages in *Nitella*. The oriented growth of the internode is thus traceable, in principle only, back to oriented events in the apical cell. The consequences of oriented expansion in cylindrical cells are more clear. In *Nitella* they include not only the typical shift in proportions seen in cell development but also an aligning action on the chloroplast files, an action which influences their growth and division and also the direction of the cytoplasmic stream.

It is thus evident that many oriented features of development may be the result of long chains of transfer of information pertaining to direction. The transfer could involve strain alignment, crystallization, or possibly other processes such as electrophoresis. The role of the genome in this aspect of development appears not to involve a simple coding of directional information but rather the specification of a complex cytoplasm capable of responding to external and internal directional "cues" (including mechanical ones) and capable of compounding this information to control the geometrical course of cell development.

Tip Growth: Kinetic and Structural Characterization

Tip growth designates the activity in a dome-shaped growth zone which typically generates a cylindrical cell surface at its base. The best studied examples are tips of pollen tubes, hyphae, and root hairs. Ordinarily the cylindrical part of a tip-growing ell does not change shape except to initiate branches. Ani exceptional secondary inflation of the cylindrical part is seen, however, in ring closure of nematode-trapping fungi. The tripling of cell volume takes 0.1 sec. Formation of arthrospares n fungi also involves a rounding up of hyphal segments.

It is sometimes assumed that the essential feature of tip growth in cylindrical cells is the restriction of surface expansion to a terminal region of the cell. Restriction *per se* is clearly not a sufficient explanation because this is compatible with growth producing a variety of shapes at the cell tip. In *Phycomyces*, for example, both the steady elongation of the tip of the cylindrical sporangiophore in the early stages and the later swelling of the tip into the round sporangium involve a restricted apical growth zone in the same cell. The particular surface behaviour needed to insure that a hemispherical growth zone does generate a cylinder at its base has been described in part by several scientists. Assuming the growth is radially symmetrical, there are three interacting parameters; shape of the growth zone, the gradient in local rate of area expansion (at different distances down the dome), and directionality of this expansion. If, for example, expansion is to be the same in all directions (isotropic as in Fig. 4.3 A) and in all regions, and if the shape is to stay hemispherical, then the gradient in local relative rate of expansion of area must decrease as a cosine function as points are examined from the tip of the dome (maximum rate) to its base (line m in Fig. 4.3 A). Rate would be proportional to the cosine of the angle (α in Fig. 4.3 B) whose vertex is the center of curvature of the dome and whose arms connect this point to the tip of the dome and to the point in question. This stems from the finding that isotropic expansion requires a point to move down the profile (m in Fig. 4.3 A) with a velocity proportional to its distance (r in Fig. 4.3 A) from the cell's axis of rotation, a sine function. The relative, elemental rate of extension of a short meridional line segment through the point would be the derivative of this, with respect to distance down the profile, a cosine function. The relative rate of increase in the area around the point would be twice this, if growth were undirected. This area rate gradient, therefore, would be a cosine function as one considered the descending rate from the tip of the dome to its base. The gradient in the rate at which small regions expand will take different forms depending upon the directed character of expansion (see Fig. 4.3).

Rate gradients within the tip growth zone. Several types of evidence indicate that the expansion rate is maximal at the extreme tip of the zone and that expansion is approximately isotropic (the same in all directions in the plane of the wall) at the tip and sometimes transverse at the base:

(a) When pairs of marks are seen along the profile of the zone, they often do not show separation from each other as they move from lower regions of the dome to the cylinderical part. Since diameter at the region of the marks does increase during this movement, expansion must be primarily along lines of cell circumference (Fig. 4.3 C). Because the extension along the meridian (profile) has ceased, and the relative increase in circumference is small, the overall growth rate at the base must be small also. Marks behaving this way are seen in hyphal tips, and on the surface of *Nitella* apical cells.

(b) The microfibrillar texture of the inner surface of such growth zones is either isotropic or slightly transverse, the outer surface being either isotropic or slightly longitudinally aligned. There is thus little evidence for well-ordered deposition of fibrils and there is only weak passive alignment of microfibrils (some of it possibly in the flattening required to make metal-shadowed preparations). Scientists report a concentric arrangement on a hyphal tip, but a random arrangement appears typical in fungi. The failure of finding morphogenetic effects of colchicine on pollen tube development also weighs aiganst a significant role for microtubules and oriented cellulose in growth of this cell type. No microtubules in lily pollen was found. These observations speak for nearly isotropic expansion, a condition coupled to a gradient from high expansion rate at the tip to low at the base.

(c) In the tropistic response, tip growth zones generally grow toward increasing concentrations of growth stimulants and away from increasing concentrations of growth retardants. Stimulants apparently bring on bulging at the very tip, in the direction of the stimulant; if growth took place primarily in subapical collars, the cell would grow away from the side rich in the stimulant. Curiously, in snapdragon pollen, Cart is a natural attractant and presumed stimulant even though in *Avena* parenchyma cells it is a "wall hardener".

(d) Autoradiographic studies show maximum incorporation of wall precursor at the very tip of hyphate of *Mucor* and Lippman. Presumably, incorporation reflects rate of wall expansion as well as synthesis.

The maintenance of a steep descending gradient in relative rate of area expansion within the apical dome appears to be the essential basis for the generation of cylindrical form in tip-growing cells. While the formation of a secondary wall in the cylindrical part could account for the cessation of expansion at the base of the dome, this is not a general explanation because the secondary wall is first clearly seen only several cell diameters below the tip in the radish root hair, and because this wall is missing in *Neurospora.*

The rate limiting process. If tip growth is maintained through a specific gradient in rate of expansion of surface, it is significant to inquire how expansion rate is controlled. It could be controlled by wall thickness, but no obvious gradient is seen in hyphae or root hairs. Given constant wall thickness, the stresses tending to expand the wall would actually be greater at the base of the dome than to tip, so the observed opposite gradient in rate must reflect complex control of wall yielding.

While in principle a wall could yield in a viscous fashion to turgor stresses without any active loosening by the cell, a concurrent lytic action is highly probable. The coordinated turning and subsequent fusion of hyphae suggest that controlled wall dissolution is a part of normal growth in fungi. The germination of walled spores and zygotes must involve local wall to being. All forms of delayed cell branching (e.g., root hair formation) suggest a weakening of the wall. Experimentally it can be shown that the cytoplasm of *Nitella* acts upon the wall to lower its yield threshold when growth is stopped structurally. The sortened wall can resume growth at reduced turgor even though wall thickness apparently increases. This suggests that in the absence of elongation the loosening action predominates over the addition of new wall mass, giving a net weakening of the wall. To account for the obvious balance between these two processes during normal elongation, the phenomenon of strain hardening (increase in strength in response to being stretched) may be invoked. It this is a property of the intact cell wall, it would counteract excessive growth rate. The preponderant softening action during osmotic inhibition may be the natural activity which promotes growth when the rate is low. Such a scheme could incorporate stability into the yielding process.

Tip growth can withstand certain perturbations of the environment (small changes in osmolarity of the medium), but in fungi the internal controls, whatever they may be, cannot accommodate large shifts in turgor. The original growth zone often becomes inextensible and cannot resume activity when turgor is restored and a new zone appears below it. The opposite effect—excessive loosening—is seen under anaerobiosis where tip-growth zones can soften to the point of bursting.

In view of the stability of tip growth, within limits, it may be an over-simplification to seek a single reaction or enzyme as the key governing factor in determing rate. The simplest functional unit of growth may be a process as complicated as the fusion of small vesicles into the wall and cell membrane.

Cytoplasmic structure. Most of the major cell organelles (mitochondria, nuclei, Golgi apparatus, endoplasmic reticulum) are relatively excluded from the dome-shaped part of the tip-growing cell. In the subapical part of a single *Chara* rhizoid, however, scientists estimate there are 25,000 dictyosomes! Golgi are rare in hyphae so the vesicles seen in growth zones presumably come from the endoplasmic reticulum. The growing region in root hairs is rich in ribosomes and, most strikingly, in vesicles of various sorts. This corresponds to cytochemical findings of RNA and polysaccharides.

Microtubules appear to be prominent only when there is a parallel textured secondary wall. They are seen in the cylindrical part of *Chara* rhizoids but are absent in many hyphae. In radish root hairs, microtubules extend further toward the cell apex than does the secondary wall. This observation opposes the generalization that aligned microtubules are associated with oriented wall synthesis. It is possible, however, that here the microtubules adjacent to the random cellulose are not yet mature enough to have an orienting role that no wall synthesis occurs in this part of the cell (between the dome and apical end of the secondary wall); or that some oriented synthesis does occur has cannot be detected in sectioned material (shadowed wall surise are more diagnostic in this regard).

Vesicles in tip growth zones. The vesicles prominent in tip growth zones a diameter of 50 to 200 mμ and appear to originate in the Golgi complex or, in fungi, in other membranous structures. Smooth vesicles, which show only a single line boundary after glutaraldehyde-osmium preparation, appear to empty their contents directly into the cell wall (also contributing their membrane to the plasmalemma) or into somewhat diffuse "fibrous inclusions" which often lack a limiting membrane Presumably, these inclusions ultimately become part of the growing wall. There appears to be a maturation of vesicle contents, including increased stainability and the development of a fibrous appearance along the apparent route of migration from the dictyosome to the wall. Other vesicles take on a coated appearance after budding from larger vesicles associated with the dictyosomes. These are also found near the cell wall, but the appearance of fusion with it is only rarely encountered. There is evidence from other systems that the coated vesicles are involved in protein transport, and in the radish root hair it has been suggested that they transport enzymes to sites of both primary and secondary wall synthesis. In rhizoids of *Chara*, the "microvesicles" (diameter 50 mμ) are abundant in the tip regions. They appear to be identical with "coated visicles" of root hairs and arise from multivesicular bodies. In hyphae, multivesicular bodies and "lomasomes" may be involved in synthesis. Similar structures appear in algae.

When the rhizoid is made horzontal, statoliths in proximal regions of the dense cytoplasm near the growing tip fall toward the lower wall. Their accumulation appears to deflect upward the path of Golgi vesicles as they move from their origin in proximal dictyosomes to their usual site of fusion at the tip of the growth zone. The deflection yields an abundance of vesicles fusing with the wall in regions unusually distant from the tip of the cell. Thus if vesicle-to-wall fusion is considered a rate limiting step in growth, the growth zone is not only made asymmetrical in its activity but is also made unusually long in the upper part of the cell. These adjustments require 5 to 10 min and precede actual curvature. It thus appears that a vesicle may containalytic enzymes to loosen and expand the wall. The same vesicle may also contribute enough mass to the wall to maintain wall thickness and strength after the expansion.

The material covered so far, the kinetics of the cell wall expansion rate, and the prominance of vesicles in the adjacent cytoplasm, suggesst certain possible inreractions. Migration (or fusion) rate could be a function of growth rate. Since the vesicles show various stages of maturation of their contents on route to the wall, variation in the migration time of vesicles might therefore automatically change the chemical nature of the materials being added to the wall. Conceivably, these variations might stiffen the wall under conditions of rapid growth (the vesicles would be immature) or soften the wall when growth was slow (the vesicles would be overripe). The accumulation of vesicles is actually visible *in vivo* in certain fungi as a *Spitzenkorper*. After a growth arrest, reformation of this body precedes any elongation—suggesting that vesicles might serve to loosen the wall only after a period of maturation. Such speculation would have more merit if the kinetics of vesicle fusion could be quantitatively related to wall expansion rate. Unfortunately, the relative abundance of vesicle fusion figures in electron micrographs does not unequivocally relate to the frequency of fusions. (It could equally well reflect the duration of the fusion process in various parts of the cell.) Awareness of the unsolved questions and complex possibilities already raised may aid in the evaluation of experimental work on tip growth.

Experimental Modification of Tip Growth

The literature on this subject reveals two large classes of experimentation. In one, an oriented factor (unilateral light, pH gradient, etc.) is throught to bear on growing or germinating cells, and a correlation with the orientation of subsequent growth is sought. In the other, a nondirected variable, either environmental (pCO_2,) osmotic pressure, unusual nutrients, etc.) or internal (a particular form of a gene) can be made present or absent. Correlations with the presence or absence of a particular cell form are obtained. Details about intermediate steps, such as altered chemistry of wall fomation, are often sought.

Experimental study of the orientation of growth. These are often termed "polarity" experiments. Experiments of this sort may be screened for data which would relate the perception (interaction within the cell) of the orienting agent to the gradient in local growth rate which is believed to characterize tip growth. With orientation by light, there are several cases which do present an intriguing parallel. Polarized light, falling vertically on the growing hypha tips of *Penicillium glaucum*, brings on elongation at right angles to the vibration plane of the light. Assuming that tip growth here involves the already described gradient in growth rate down the dome-shaped growth zone (rate decreases as a cosine function as points farther down the dome are examined, as in Fig. 4.3 A), a direct parallel between local rate of growth and the amplitude of light absorbed is possible Oriented dichroic photoreceptors in the cell cortex are typically involved in the response of spores to polarized light. If in the cortex of these hyphae such photoreceptors were oriented parallel to has been surface, then in the horizontal plane at least there would be; fall-off in the relative amplitude of light absorbed (as a function of distance down the dome-shaped cell) which would also decrease as á cosine function. Intensity would decrease as cosine. Local growth rate, in light could thus be a simple function of the amount of light absorbed. A similar relationship could be present in the chloronema of the moss *Funaria*.

There is considerable evidence that many orienting agents active as spores or zygotes do not work directly through a gradient in their interaction within the cell, as could be the case above. Rather, the interaction a highly amplified to give the response, or, to put it another way, the applied influence acts on a metastable cell cortex to trigger a growth response. The evidence follows: (a) In the action of light gradients on germinating cells increasing the steepness of the gradient improves the degree of statistics orientation of the outgrowths, but does not obviously alter the size or shape of the germinating structure. This is true when the gradient is light working on zygotes of *Fucus* or a diffusing agent working on germinating *Botrytis* spores. The orienting agent the appears to contribute little in the way of energy or information needed for protrusion formation *per se* but merely signals where the protrusion will be made. The fact that germination can occur in the absensence of the experimental orienting agent supports this view. (b) The area of the outgrowth intially involves only a few per cent of the cell cortex. The gradient of the absorption within this small area would probably be too small to be effective. Presumably, the intensity gradient across the whole cell is perceived, then the protrusion is triggered at a low point in the gradient. The equivalence of oriented response between half-illumsated cells and cells illuminated with plane polarized light strongly indicates that at least 90° of the cell perimeter is involved in perception, even though only a small fraction is evolved in the response. (c) In *Botrytis*

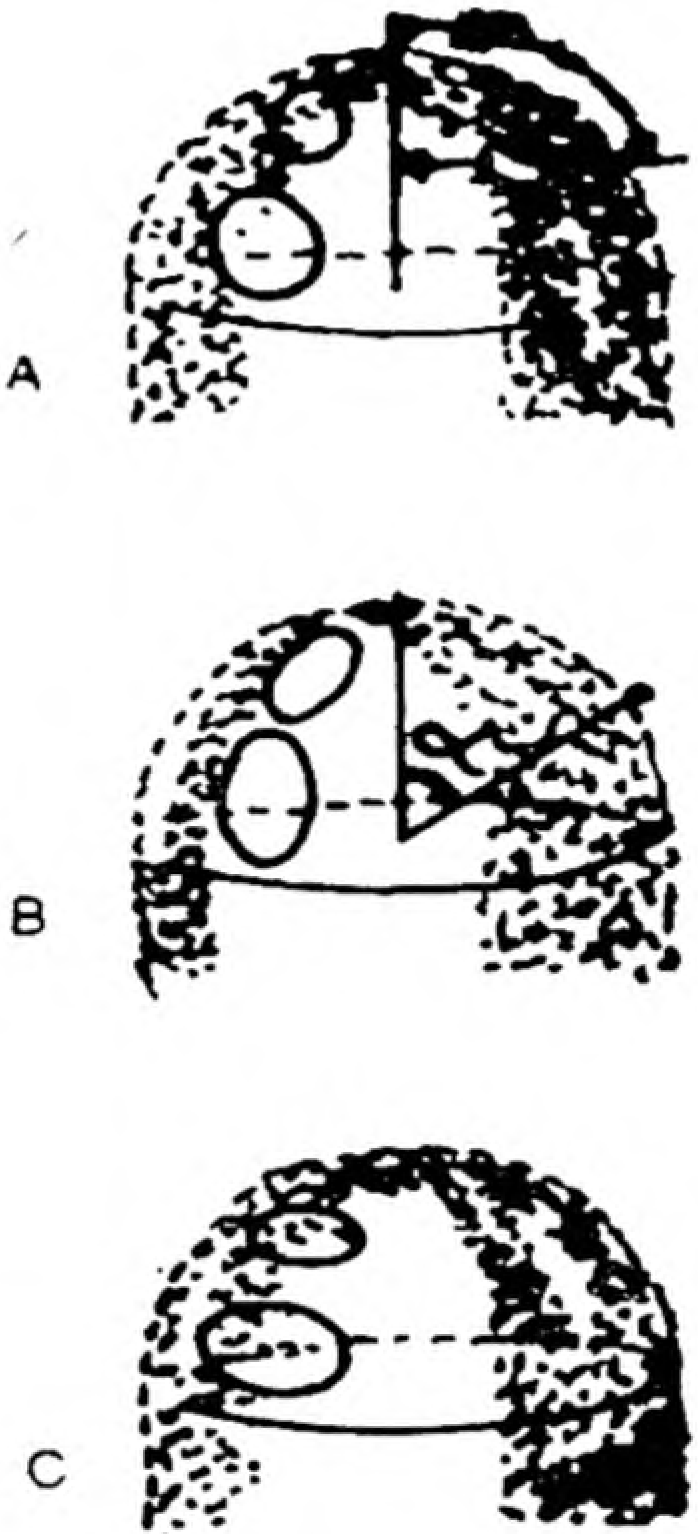

FIG. 4.3: The various modes of surface enlargement possible in a hemispherical growth zone. Depending upon the pattern of the velocity of a small area moving down m, in relation to its distance r from the axis of rotation, a small area may be enlarged without distortion as in A, extended longitudinally as in B, or extended transversely as in C. Some hyphae and the Nitella apical cell behave as in C. Most tip-growing cells behave approximately as in A. (After Green and King).

spores subjected to intense unilateral illumination, the outgrowth often appears midsary between two regions where it would be expected on the basis of the response to weaker to light. This appears to reflect summation, in this region, of influences from the two centers. Here again the geometry of the protrusion itself is quite restricted compared to the large regions of the cell cortex involved in preception.

Scientists have shown that a diffusion macromolecule may be involved in the control of oriented germination in *Botaytis*.

When subjected to show flow of the aquatic environment, the stationary spores germinate downstream, the direction in which the molecule would accumulate. If this same molecular gradient were essential to continued growth, the absence, not presence. of the molecule would have to correlate with high growth rate because the environment of the hyphal tip pushing through the medium would be equivalent to the upstream side of a stationary but unilaterally irrigated spore.

While gradients of light and of large molecules seen in flow studies are active in orienting germination, their direct involvement in the physics of tip growth is considered unlikely. Electrical currents and ion gradients, however, appear not only to act to orient germination but may be involved in the normal tip-growth mechanism as well. Has shown that an electrical current flows through the germinating *Fucus* zygote as the protrusion (rhizoid) elongates. The current might suffice to concentrate particles in the size range 00.1 to 0.1. at the rhizoids tip. Golgi vesicles might be influenced. *Fucus* eggs was oriented by potassium and hydrogen ion gradients. It was concluded that the gradients act by establishing a voltage gradient across the cytoplasm. In general terms, it is possible that the current, through an electrophoretic action on cell contents, promotes the process which causes it. These general possibilities are reviewed. On the basis of turning maneuvers seen in hyphae tips, (Scientists) propose that hyphae continuously emit a growth retardant (to explain the "repulsion" of an approaching tip by a mature hypha) and also remove in locully at the very tip (to explain the "attraction" and fusion sometimes seen between two hyphal tips). The question of what maintains the gradient in expansion rate in tip-growth zones contains the subquestion of whether the basic mechanism includes camponents outside the cell membrane (but possibly in the cell wall, as in aerial hyphae). The above studies suggests that they do.

Experimental Study of the form of tip-growing cells. Eexperimental variables in this category do not cantain an oriented or vectorial component. They include genetic, nutritional, and environmental factors.

Genetic analysis. The chain of causation between gene and morphological expression in tip-growing forms can be approached from the gene end by the analysis of mutations. In *Neurospora* about 100 morpho logical mutants are known. These differ from the traditional metabolic mutants to being relatively autonomous: they are less subject to modification by environmental factors, and the deficiency is not usually restorable by an expaneous supply of gene product, although exception will appear below.

Certain poorly growing mutants have been shown to be non-nuclear in nature, the character being transferrable by cytoplasmic injections. An abnormal type of mitochondrion, replicating more rapidly than the normal, appears to be the effective agent.

In a thorough investigation of a nuclear mutation more closely related to form several Botanists showed that the earliest identifiable gene product associated with an abnormal mycelial type was an abnormal form of glucose-6-phosphate dehydrogenase (G6PDH). The mutant, *cot*, had slow-growing dense colonies with bulbous cells rich in branch points; it had a form of the enzyme that was more heat labile and that had a different affinity for substrates. Strains with this form of the enzyme invariably had high levels of glucose-6-phosphate (G6P). Genetic reversion of colony form coincided with a reversion to the normal form of the enzyme, indicating that the *col* locus is the structural gene for the enzyme. The same colony form could be found, however, with wild-type nuclei, provided they were grown on sorbose. Other nuclear mutations give the *col* growth form but not the mutant form of the enzyme, as judged by the normal level of G6P. This shows that the *col* growth habit does not cause the abnormal enzyme but also that other genetic and nutritional variations lead to the more morphological peculiarity. It is likely that some late step in the corbohydrade chemistry of the cell wall (perhaps only indirectly influenced by levels of G6PDA) is altered by the diverse treatments that can lead to the *col* morphology. Scientists found that one cell wall fraction (one containing glucan, galactosamine, and amino acids) varied in *col* relative to normal mycelia. The direction of variation form normal was, however, not consistant. Analyses were of whole mycelia rather than the growing regions. Later it was shown that this form-related chemical fraction comes from a outer wall layer of thick fibrils. This layer might be wall present in the growth zone. An inner layer contains thin fibrils of chitin.

Generally, difficulty of analysis of a mutation increases rapidly with greater distance (in terms of steps) from the gene. In related work on the Basidiomycete *Schizophyllum*, Scientists have corrected the activity of a wall glucan degarding enzyme with the operation of one genetically determined developmental sequence (alternation in septum structure and stability) governing dikaryon formation. In the same genus, cellobiose (but not maltose) induces wavy and highly branched hyphae. Cellobiose derepresses a glucanase which appearntly lowers the proportion of a β-1, 3 glucan in the wall. The link between these chemical-enzymatic studies and the physical nature of tip growth has not been elucidated in detail.

Interconversion between filamentous tip growth and budding. Johnson and Gibson have shown wall precursors to be incorporated primarily in one growing end in fission yeasts and in the distal region of buds in budding yeasts. Assuming this incorporation (into β-3 glucan) reflects surface expansion, many fungi seem to show localized growth. Certain mutant forms of the yeast *Candida albicans* show filamentous rather than budding growth. Normal and mutant growth behaviour is often described by the contrasting terms "dividing" and "divisionless". Much of the literature on this "dimorphism" implies fundamental differences in growth mechanism, including a difference in the chemistry of the rate-limiting reaction in the cell wall.

The two modes of growth appear, however, to be very closely related and can give about the same rate of growth. A budding cell can bud repeatedly at or near the same end, so it would appear to involve only a minor modification in growth pattern to have periodic budding, at the same relative position of a cell, give rise to a beaded filament. As the periodic nature of budding become continuous, the filament would lose the periodic bulging appearance and resemble a hypha. All of these intermediate forms are seen in the mutant strain when it is cultured on the same agar with the normal form. The local expansion behaviour of the growing surface in the two forms has not been described.

The diffusing factor which restores budding to the filamentous mutant appears to involve reduced organic sulphur because it can be replaced by cysteine or reduced glutathione. The filamentous form can reduce dyes vary effectively (more so than the normal strain) but seems unable to carry out some reduction; presumably involving sulphur, needed for budding. In *C. albicans*, Nickerson has shown that the hyphal form has a lower level of an enzyme, protein disulphide reductase, than is found in the yeast form. Moreover, the wall contains a gluco-mannan-protein complex rich in sulphur which is partly oxidized to disulphides. While the enzyme is found in mitochondria and the assay involves previous chemical oxidation of the protein in the wall, it has been suggested that wall softening by enzymatic reduction of the disulphide is the essential basis for budding. How the wall softening is accomplished in the filamentous form, which grows at the same rate and which also contains gluco-mannan-protein in similar amount, is not clear. It seems at least equally reasonable to suggest that the differences in sulphur metabolism between the two strains deals not with a fundamental difference in the nature of the rate-limiting reaction of surface expansion, but rather with the control of the spatial distribution of a yielding process which may be identical in the two forms.

Yeast-like and mycecial forms of *Histoplasma* and *Saccharomyces* can be produced by nutritional variations. The yeast-form walls were relatively high in chitin but low in mannose and amino acids. On 17 amino acids detected, only methionine (trace amounts in both) contained sulphur. The keratin-like protein of *C. albicans* was appearently absent. The wall of *Saccharomyces* appears to lack sulphidrils The glucan-galactosamine-peptide in the wall of *Neurospora* appears to lack sulphur Thus, few tested cell types appear to contain wall structure with the disulphide bonds and sulphidrril groups needed to give a protein disulphide reductive a possible general direct role in wall alteration. The assays may, however, suffer from breakdown of sulphur amino acids during hydrolysis.

In the mold *Mucor*, a conversion of the typical filamentous elongation to a budding growth habit can be brought on by high concentrations of CO_2. A clear and well-illustrated review is provided by Bartnicki-Garcia in 1973. Concentrations of CO_2 (pCO_2 of 0.3 atm or more) are most effective if oxygen is excluded from the mixture. Pure nitrogen yields the filamentous form. The morphogenetic effects correlate well with the actual uptake of CO_2, presumably by the malic enzyme whose optimum pCO_2 is near the peak concentration for the development response. The main product is a 4 carbon dicarboxylic acid. The major chemical difference in wall composition between the two forms involve greater mannan and protein in the yeast form. It is suggested, therefore, that the CO_2 fixation enhences mannan-protein production. While yeast-form walls appear thicker (and double-layered) in the electron microscope, the over-all wall synthesis is roughly comparable on a weight basis. It is not clear whether the difference in mannan and protein, found for whole walls, also characterizes the small fraction of the wall undergoing expansion. The metabolic alterations bringing on budding may therefore involve the control and distribution of the expansion process, rather than a drastic change in its chemical nature. This is supported by the highly localized incorporation of radioactive wall precursor in the tip of hyphae, compared to a more scattered pattern in the yeast form.

In related yeast, Scientists have shown lysis at the growing region to be brought on by 2-deoxy-glucose. They suggest that rupture occurs because this glucose analogue interfers with the intercalation of new residues in the midregions of glucan chains. All cell wall polymers are believed, however, to grow at one or both ends, not in the middle, so matrix substances are probably involved.

Origin of Tip Growth

Tip-growing cell surfaces commonly originate from spherical spores of zygotes, from the sides of cylindrical cells, or, in the remarkable case of the desmids, from the cross-wall of the previous cell division.

The maintenance of tip-growth involves the perpetuation of certain types of gradients in local expansion rate down the dome-shaped growth zone. The origion clearly involves the initial establishment of such gradients. Very little kinetic data appears available for the conversion of a flat area into a hemisphere. This phenomenon has beed only crudely explored in simple models. A rubber membrane, fixed like a drumbead over a cup, can be deformed into a near-hemispherical shape by evacuating the cup. Markings on the membrane show that when the membrane is of uniform thickness, there is a gradient in amount of isotropic expansion from the centre to the rim, the maximum being in the centre. When the membrane initially has a thickness gradient from the centre to the rim, the deformation is no longer isotropic because circles drawn on the membrane are pulled out along lines of longitude on the dome-shaped structure. A thickness gradient falling from the rim to the centre gives the opposite effect: circles are pulled out along lines of ltitude. This latter effect, which is actually observed in the initial swelling of leaf apical cells in *Nitella*, could serve to align microtubules into the transverse orientation, an alignment which they maintain in cylindrical cells of this genus. The coupling between a gredient in yielding rate and a specific orientation of distortion of surface in a swelling structure may be of some significance in the generation of oriented textures in biological systems.

Two major unanswered questions about the origin of tip growth appear to be:

(a) what initially delimits the base of the swelling; and

(b) what ensures that the swelling does not balloon up into a vesicle (bud) but rather extends as a cylinder with a diametre about equal to the base of the swelling.

Origin from the spore or zygote. This subject was discussed under the control of tip growth by oriented aspects of the environment. Most recently has been shown that the electric current which approximately come with the appearance of the rhizoid in *Fucus* actually precedes the protrusion in the germination of *Pelvetia.* This current may be an intermediate between the light stimulus which orients development and the physical manifestation of growth.

Origin from the sides of cylindrical cells. The most studied process of this sort is the origin of the root hair which, in grasses, occurs just prior to the cessation of elongation of the epidermal cell. Avers and associates have applied cytochchemical tests for various kinds of enzymatic activity in prospective hair-bearing and hairless cells. In some grasses these cell types are identifiable after an asymmetrical division in the young epidermis; in other grasses a prediction is not possible. Their survey, limited by the availability of tests, did not discover any onr enzyme universally correlated with root hair production. In the class with the asymmetrical division, some enzymes appeared prominent in the prospective hair-bearing cell while at least one enzyme appeared relatively excluded.

The role of calcium in the initiation and growth of root hairs has been reviewed and debated. Ethylene definitely promotes the formation of root hairs. At the same time, it brings on the production of relatively isodiametric cortical and epidermal cells. Whether the promotion of initiation of tip growth is based on the conditions of the wall or the unusual proportions of the cell (presumably brought on by the previous condition of the wall) is unknown.

Origin in the cross wall. In the desmids, the new cross-wall splits into two lamellae, one for each daughter cell. Each such lamella becomes, in effect, a primary wall which than expands enormously to form an entire new semicell (Fig. 4.4). A cross-wall thus becomes a side wall. The opposite relation occurs in cogai where a equalized band on the side of the cell grows in so as to "pinch" the cell into two. Scientists have recently reviewed this morphogenesis in desmids. Mutants almost certainly of a cytoplasmic sort because they can be formed in relatively high yield by mild techniques such as cold treatment and centrifugation, lack one or two lobes of the three that characterize each semicell. It has been postulated that three cytoplasmic "axes" pass through the isthmus and govern the formation of the lobes. Mutation is thought to be the loss of an axis and reversion to be its restoration by duplication of an unaffected axis and the subsequent relocation of the extra axis to re-establish normal form. Attempts to find structural evidence for the axes, however, have yielded little. The future lobes appear to have their earliest manifestation in the form of thichness variations in swollen form of the cross-wall which appears when growth is osmotically inhibited. Thin regions in it correspond to prospective major indentations between lobes. These minima correspond to attachment-sites of the plasmolysed protoplast, suggesting that at least the main outlines of cell form correlate with cell membrane properties immediately after division. The wall appears to be semifluid and the indentations

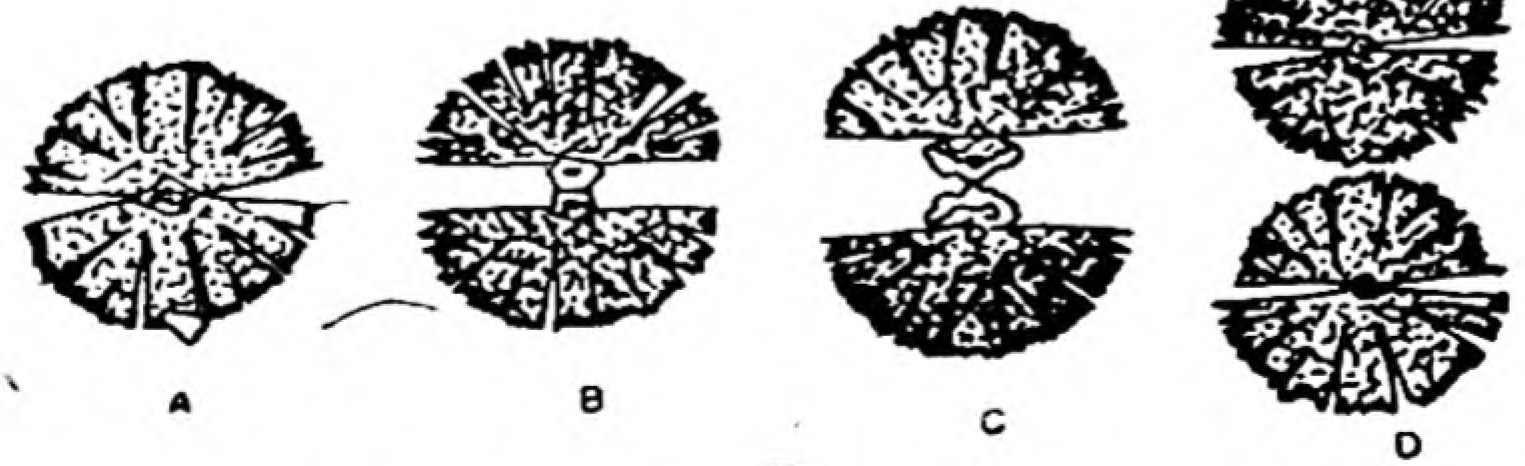

FIG. 4.4: Sequence of stages in the formation of new semicells in desmids. (After Waddington)

of the septum can be seen to suddenly "evert" smoothing the outline of septum. Kiermayer suggests that this results from the breakage of certain cytoplasmic strands which can be seen penetrating the wall, particularly at thickness minima. Upon return to normal turgor, the wall is compressed nearly to its normal thickness, but subsequent development is abnormal. While some growth does occur along lines between indentations and the isthmus, most extension occurs in to lobes.

Although excellent time-lapse photographs of semicell formation exist, they do non permit analysis of local growth rate within the lobes. The lobe tip, with its apparent greater attachment to the membrane, may be either a rate minimum. or a maximum Bifurcation of the lobes appears promoted by high nuclear/cytoplasmic ration and is prominent in polyploid forms.

In sepra developing at some distance from a nucleus—due to experimental manipulation—the degree of bifurcation is less than normal. The semicell form in growing anucleate cells may consist of only the major lobes in simple outline. While this may reflect loss of a direct morphogenetic influence of the nucleus, it may be a side effect of low turgor in the enucleate cell. Low turgor in general leads to simplified cell outline.

MORPHOGENESIS IN CELL CULTURES

The basic assumption of cell and organ culture as an experimental method is that many of the interactions essential to organismic control of cell behaviour can be sorted out and made to occur independently of each other. The first step consists of working with "cuttings", *i.e.*, isolated organs or organ systems. When this is done under sterile conditions, the method is referred to as "organ culture." Organ culture is not reviewed here since its methods and results require separate consideration in conjunction with the many unrelated problems which have been studied by this means with such notable success. In contrast, "cell culture" hangs together as a coherent subject susceptible to review because of its few succeses and its concentration on technical problems. Included in the term cell culture are the following: "callus" (clumps on static media), "suspensions" (single cells and clumps in agitated liquid media), "plate cultures" (individually identifiable single cells and clumps on agar plates), and "microcultures" (one or several cells grown in chambers which facilitate microscopic obesrvation). The term "tissue culture" has traditionally been used as an inclusive name for both organ and cell culture.

Cell culture may be regarded as the ultimate step in sorting out interactions, since the raw material consists of a disorganized population of cells in which normal histological patterns are suppressed. or allegedly so, until the proper environment is introduced for invoking selected morphogenetic processes. Current techniques emphasize the application of suspected morphogenetically active chemicals to hopefully totipotent cells. The difficulty with this approach is that it overlooks the importance of physical factors and also the crucial difference between totipotence and competence. The capacities of individual cells in culture are usually unpredictable. Thus, while we have made advances in identifying chemical stimuli which evoke a particular morphogenetic response, the cells which are competent to respond are few in number and are generally not identifiable in advance. Elucidation of the nature of competency has been overlooked since the identification of chemical morphogens requires nothing more than the observation of a limited morphological response to alterations in the nutrient medium.

Throughout this review, the terms *differentiation* and *morphogenesis* will be used in the traditional imprecise way, the former referring to chemical and structural changes in individual cells as they depart from the features defined as eumeristematic and take on other specializations, and the latter to any and all morphological changes, subcellular or supracellular. The precise definitions of these terms, which have emerged from microbial genetics and biochemistry, are not yet applicable to multicellular organisms.

A painstaking review of nearly all aspects of plant tissue culture prior to 1959 may be found in Gautheret's monumental *La Culture des Tissus Vegetaux*. In addition, a number of excellent reviews dealing with various aspects of morphogenesis in cell cultures have appeared later on. Accordingly, no attempt has been made here to review the literature exhaustively. Selected problems are discussed, primarily from a methodological standpoint, with emphasis on cytological rather than physiological data.

Primary Cultures

Primary cell cultures, *i.e.*, those established directly from the organism, ideally should consist of one cell type with well-defined development potentialities. Theoritically, this result might be achieved in at least three ways:

(a) short-term culture of a population of progenitor cells committed to a known fate;

(b) culture of specialized cells which retain their differentiated features through successive mitotic cycles;

(c) establishment of a meristematic cell population uncommitted to any course of differentiation but capable of many.

Progenitor cells. A prime example of the use of progenitor or "blast" cells in following the final stages of differentiation *in vitro* is the well known work on myogenesis and chondriogenesis with animal tissue cultures. With the notable exception of meiocytes, embryonic cells which are the equivalent of blast cells cannot be obtained from plants.

Specialized cells. The inevitable question arises concerning the ability of cells to re-enter the mitotic cycle and retain specialized functions. In a recent review in this series, Heslop-Harrison provided a lucid discussion of the evidence from cell cultures which bears on this problem. He concluded that the transmission of differentiated states by single cell cloning had not been demonstrated. Retention of such states through single cell cloning would eliminate the possibility that they were dependent upon interactions between cells, a situation which has been referred to as "group stability."

The recognition of differentiated states depends upon three properties of cells: morphological features, physiological or chemical features, and latent capacities. Characteristic morphological features are not likely to survive repeated cell divisions, although there are exceptional cases. Tulecke reported, for example, that cells in callus derived from *Taxus* pollen grow "in an elongated manner typical of growing pollen tubes", with the nucleus and cytoplasm located at one end. On the other hand, Scientists followed the cytological changes accompanying a renewal of cell division *in vitro* in palisade cells of *Arachis hypogea* and observed that "no derivative had any morphological resemblance to the original palisade parenchyma cell." Whether or not the derivatives retained any physiological features which might be peculiar to palisade cells is an open question.

Where good chemical criteria exist, as in animal cells, it has been possible to demonstrate the continued synthesis in long-term cultures of tissue and organ-specific products. In at least four cases, the cell strains were derived from single cell clones. Surprisingly enough, we know very little about specific products of specialized plant cells. The production of alkaloids and other secondary compounds occurs in some cultures, but their synthesis in the intact plant normally occurs in meristem and is therefore not a differentiated function in the sense of belonging to cells outside the mitotic cycle. A special case of stable chemical differentiation may be seen in the tannia cells which "breed true" during both normal ontogeny and induced regeneration in *Ricinus*. Bloch interpreted the arrangement of such cells as evidence that single differentiated initials divided to yield identical progeny. His attempts to culture such cells were unsuccessful for technical reasons, but he did conclude that they were irreversibly differentiated. Scientists observed similar cells reproducing true to type in *Sequoia* callus, but found some evidence that they dedifferentiated when incorporated into odd primordia.

Examples of the retention of latent capacities may be found in the studies of European workers on *in vitro* induction of flower buds in vernalized or photoinduced cuttings, and studies by Bauer on regeneration of mosses. The flowering studies of interest are those where, following induction, a considerable period of callus growth ensued before floral primordia were organized. A particularly striking example of this was the formation of flowers in tumors induced by injecting *Agrobacterium tumefaciens* into the internodes of *Nicotiana tabacum*. However, in all the above cases, the buds were formed in callus still attached to the parent cutting, and the influence of the remaining mitotically inactive tissue is uncertain. The logical extension of this kind of experiment, namely subculturing of the callus, has been performed by Bauer in his unique studies of regenerating moss sporogonia. Explants removed from young sporogonia regenerated in different ways, depending upon the region of excision. Some segments gave rise to protonema which in turn produced leafy plants, or protonema which produced sporogonia directly. Other segments yielded callus which remained unorganized in the light but produced sporogonia in the dark. Under the proper conditions, the sporogonial factor was stable through a number of subcultures. Other cases are known of stable morphological or metabolic features which arise only after callus has been subcultured for a period of time and which cannot be referred to some aspect of the tissue of origin. These may be of no relevance for understanding normal mechanisms of differentiation, since stability of this kind probably arises from selection of a mutant population.

None of the above cases of stable differentiated states have been subjected to single cell cloning, a critical step as pointed out by Heslop-Harrison. Since single cells of both lower and higher plants can be cultured, it should be possible to perform more critical analyses. A theoretical basis for clonal transmission of the differentiated state exists, namely the "paragenetic" mechanisms for gametic transmission of the functional state of particular gene oci. In the case of higher plant cell cultures, however, the issue will be confused by the considerable variation within secondary clones derived from a single parent cell.

Meristematic cells. The ideal situation would be a primary culture of meristematic cells which can be induced on masse to yield specialized cells or organ primordia of one type. However, it is doubtful that a uniform population of meristematic cells can be obtained directly from the plant. The diversity of structural types within a single meristem has been emphasized. More significantly, studies of the periodicity of division in roots led to the conclusion that there is no meristematic cell existing "as a distinct cell type whose function is primarily the production of new cells. . . ." This conclusion was based on the finding that mitosis was a property of cells in radial and longitudinal enlargement in a region where tissue patterns had already been blocked out. This suggests that if it were possible to dissociate meristems and obtain a population of meristematic cells directly, one might have a mixture of subpopulations which were the functional equivalent of different kinds of blast cells. Regardless of the validity of this argument, it is not possible at present to dissociate plant tissues in the same way as animal tissues by enzymatic means or by chelation, although recent work with hydrolytic wall enzymes in obtaining living protoplasts suggests that the technical problems may be overcome. In practice, cultures have been started by inducing cell division in relatively massive explants consisting largely or entirely of cells which have ceased to divide as a result of normal maturation. The first steps in growth of such cultures involved a series of regressive cytological changes referred to as differentiation or embryonization. It remains to be seen whether or not it will be possible by this method to produce a uniform population of meristematic cells.

Dedifferentiation. The term embryonization has been preferred by some workers to describe this process since it does not imply a loss of functional specialization. There is some justification for this view since as discussed earlier, certain differentiated functions of the primary explant may persist through considerable subculturing. This may be the rule rather than the exception for animal cells. However, the term dedifferentiation in most cases of the renewal of growth in mature plant cells accurately describes a fundamental alteration in the differentiated state, since the daughter cells commonly show the ability to redifferentiate into cells of another type than the parent.

Buvat's early investigation of the renewal of growth in mature cells from a variety of tissues and organs remains the most extensive to date. The limit of the dedifferentiative process is reached when, by virtue of division proceeding more rapidly than enlargement, small, densely cytoplasmic (eumeristematic) cells are produced. However, Buvat found that dedifferentiation involved at least one intermediate stage beyond which certain kinds of regressing cells could not, or at least did not, pass. According to later workers, this phenomenon of partial dedifferentiation was characteristic of young lignified cells such as fibres, collenchyma, or of immature vessels and immature sieve tubes. Gautheret emphasized that the subsequent morphogenetic potential of such partially dedifferentiated cells is restricted to histogenesis, whereas cells which are derived from parenchyma, companion cells, secretory cells, or cambium origin a potential for organogenesis. It is not clear whether the inability of certain cells to dedifferentiate fully in the studies cited above was the result of a permanent block, possibly genetic, or of other factors. The fundamental implication of these findings requires that they be substantiated in an unambiguous way.

It is evident that a complex explant necessarily gives rise to a culture initially consisting of an extremely heterogeneous mixture of cell types. The difficulties inherent in the use of such material are obvious. Nevertheless, a considerable number of important generalizations have emerged from studies of the histological changes occurring during induction of growth in complex explants. For a detailed discussion of all aspects of this work, the leader is referred to the excellent reviews by Gautheret. In addition to these histological studies, there is a vast background of work on the physiology of newly excised tissues. These data cannot be covered here and will be discussed only in so far as they bear directly on subsequent morphogenetic events in established cell strains.

Differentiation

The majority of cells in any culture have gross cytological features similar to thin-walled ground parenchyma of the intact plant, but the resemblance does not include a regularity of shape. A variety of shapes, many quite bizarre, occurs due to random yielding of wall areas and irregular orientation of cell plates. The significance of this fact for classical concepts of growth and cell division has been discussed in considerable detail by Steward. In addition, cells of *Picea* in culture show an unprecedented range of dynamic activities, including the ability to extrude a variety of large drops, strands, fibres, etc., through the cell wall. Whether or not these activities are representative of normal processes which go unobsered in the intact plant is uncertain, although white believed this to be the case. The greatest interest naturally attaches to definition of the circumstances under which irregular cell growth is succeeded by normal patterns of growth and differentiation. The reassertion of such patterns is usually restricted to the development of cambia, vascular tissue, and organs.

One difficulty in interpreting the circumstances necessary for normal growth arises out of the fact that the same substances which are required to initiate and maintain callus growth are also implicated in the initiation of organised structures. While this has obvious significance, it is undesirable experimentally. Thus when one finds the statement that a particular culture was "undifferentiated" at the start of an experiment, one usually has to revise this mentally to read "no organs or other structures were visible to the naked eye." Unorganized eumeristematic loci which can be carried through a number of subcultures have been described by Gautheret as "meristemes degrades". One example is the proembryogenic mass which can be present unnoticed in cell cultures and will not produce an embryo until the auxin level is lowered. In addition, organized primordia which cannot develop further because of inhibiting auxin levels may be present. It is clear that assumptions cannot be made about the role of growth substances or metabolites in organogenesis or cell differentiation unless a thorough histological study of the culture has been made before the experimental treatment begins. This is seldom done.

Cambia

Gautheret noted that the circumstances surrounding cambium development in cell cultures were fundamentally similar to those in intact plants. He concluded that "cambiums naissent toujours a la limite de deux tissues de nature different." The major types of cambia found are the phellogen-like tissues which arise where parenchymatous cells border the periphery of the explant or callus, and the vascular cambia which usually arise in relation to pre-existent tracheids or seive tubes. The cell types produced by vascular cambia vary with the origin of the primary explant and the nutrient medium. According to Gautheret, the functioning of newly formed cambia is initially "conditioned by the nature of the surrounding tissue." Thus, cambia regenerated at the periphery of an explant will produce phloem towards the interior and xylem towards the exterior if the explanted tissue consists of phloem, whereas the reverse orientation occurs if the explanded tissue is xylem. Aspects of this kind of directed differentiation, known as homeogenatic induction, are discussed in some detail by Lang. The data from cell cultures and other experimental systems have been used by Wilson and Wilson in their "gradient induction", hypothesis, which attempts to account for both the locus at which a cambium originates and its orientation with respect to production of xylem and phloem. Also of relevance are the ingenious in *vitro* experiments of Brown and Sax on the role of mutual cell pressures in cambial function.

Vascular Tissue

Few reports in the literature of cell culture fail to mention the random differentiation of xylem, indicating that the conditions necessary for differentiation of tracheary cells are easily met under culture conditions. Phloem is less frequently reported. Whether this is due to more exacting requirements for sieve tube differentiation or to the difficulty of detection is uncertain. Recent work favours the latter interpretation. The cases with which we are most concerned are not the random observations but those in which systematic attampts have been made to control vascularisation.

Chemical control of vascular tissue differentiation. In the 1950's Jacobs and his co-workers demonstrated a quantitative relationship between indoleacetic acid concentration and the differentiation of tracheary cells in both wound regeneration and normal ontogeny in *Coleus*. Subsequent work with the same experimental system established a similar relationship for sieve tube regeneration. French workers had earlier observed that buds regenerating in callus or grafted into cellus or cuttings induced the vascularization of subjacent tissue. Genus found that auxins could, to a certain extent, replace the bud effect. Wetmore and his associates subsequently showed that the effect of buds grafted into callus of *Syringa* was fully replaceable by sugar and auxins, although they were unable to observe in their system the correlation between auxin concentration and quantity of vascular tissue as described for *Coleus* stems. The sucrose concentration determined to amount and type of vascular tissue formed, low sugar favouring xylem, high sugar favouring phloem, and intermediate levels favouring a balance of the two.

The response of *Syringa* to sugar may be a general one, since similar results were found with other species. Jeffs and Northcote found that vascularized nodules (associations of phloem, cambium, and xylem) were induced in *Phaseolus* callus only by "disaccharides which contain an α-glucosyl radical at the nonreducing end." They cited the work of Van Lith-Vroom *et al.*, as further evidence of the same phenomenon. However, the histological studies performed by the latter workers on differentiation in crown gall cultures of *Nicotiana tabacum* seem more contradictory than supporting. Growth of the tissue on glucose produced large vascularized nodules, complete with phloem, xylem, and cambia, whereas on starch media (which presumably yields maltose, an α-glucosyl disaccharide) the cultures had "a parenchymatic character with few and very scattered lignified elements." Ball found that the most effective sugars in including vascularized nodules in *Sequoia* callus were a monosoccharide (raffinose) and a β-galactosyl disaccharide (lactose). Although other differences in the media may have played a role in these conflicting results it appears that the effect of α-glucosyl disaccharides on *Phaeolus* may not be a general one.

In some cases, a large number of individual taacheids are induced, apparently unassociated with cambia or phloem. According to Bergmann, kinetin increases the general level of lignification in parenchymatus callus cells of *Nicotiana* and also the percentage of tracheids. Chemical analyses showed that kinetin promoted lignification at the expense of other wall components by shifting carbohydrate metabolism into the pentose phosphate pathway, thereby promoting synthesis of the phenylpropane precursors of lignin. A similar inductive effect of lignin precursors on xylem differentiation was proposed by Jensen on the basis of auxin-induced proxidase activity in roots of *Vicia faba*. However, Fosket andd Miksche have argued that lignin precursors are not likely to be involved in the control of secondary wall patterns, citing evidence of other that xylem elements differentiate before detectable lignification occurs. Geissbuhler and Skoog made essentially the same point 10 years ago in discussing the illogicality, in the absence of chemical data, of referring to the differentiation of tracheid-like cells as "lignification." Unfortunately, this is still a common practice. In view of the multical physiological effects of kinins, there is no need to assume that the chemical and structural differentiations are caused by the same mechanism.

Since all the substances shown to influence vascular tissue differentiation have been hormones or metabolites which play a basic role in cell growth and division, considerable uncertainty exists with regard to the specificity of such substances in particular pathways of differentiation. Unequivocal separation of the growth function and the possible inductive function has not been accomplished.

Cell division and differentiation. The most controversial conclusion reached by Wetmore and his associates was their interpretation of the conditions necessary for cell differentiation. They state that "whether there is an elongation phase of little or great extent, that is, whether there is a procambial phase or not, the inuduction of phloem and xylem can happen if and whenever the necessary biochemical agents are available in appropriate concentrations." This hypothesis is obviously at odds with the concept gained from studies of both pland and animal cells, that the ability of a cell to differentiate in a particular way is a temporal affair which comes and goes. For example, Shininger has recently shown that decapitated *Xanthium* plants will continue to form relatively normal secondary xylem if the cut end of the stem is fed naphthalene acetic acid. If application of the auxin is delayed, the cambial derivatives which form in the absence of auxin will remain undifferentiated regardless of subsequent auxin applications. This result suggests that the period of competence had passed (although the possibility of inadequate translocation of the stimulus cannot be overlooked). Of equal significance is the fact that corrot culture consisting entirely of proembryogenic cells never contain vascular tissue although they are grown in the same nutrient medium on which control cultures not containing proembryogenic cells form the usual vascularized nodules. Wetmore and his co-workers based their hypothesis of the nonessentiality of procambium in the subsequent differentiation of xylem and phloem on the assumption that the only role of the procambium is to block out the shape of the stele. Yet they recognized that in callus the cells at the centre of the actively dividing nodules were "biochemically favourable for induction and differentiation of vascular tissue." On the strength of their own observation, it can be argued that the divisions in nodules yield cells which are the physiological equivalent of procambium, although there is no obvious morphological resemblance and that this physiological state is essential to subsequent overt differentiation of xylem and phloem. The argument for the nonessentiality of procambibm would seem to require the demonstration that mature parenchyma cells can be caused to differentiate into vascular cells without an intervening mitosis. There have been reports that would xylem may differentiate from parenchyma cells by direct transformation, but a completely convincing documentation of this point does not seem to exist.

In a recent carefully controlled study of differentiation in stem segments of *Coleus*, Fosket concluded that "cells must divide in order to intiate would-vessel member differentiation." Jeffs and Norther, using the experimental techniques devised by Wetmore and Rier, also concluded that "mitosis immediately precedes differentiation." Recent studies with differentiating animal cells led to the conclusion that immediately prior mitosis were frequently essential to expression of a particular phenotype. Apparently in some cases there are molecular events essential to subsequent differentiation which can occur only during or immediately following mitosis. This kind of interpretation is not new, since it was hypothesized some time ago that the florigenic stimulus was not effective unless it arrived an apex where cell divisions were occurring.

Organogenesis

The evolution of hormone and nutritional hypotheses in organogenesis has recently been reviewed by Dore, including extensive data from cell and organ cultures which bear on this problem. It is only necessary bere to review the methodological advantages and disadvantages of cell cultures in studying organogenesis and to discuss recent contributions of basic interest.

The structural polarity of organ formation in intact plants and in regenerating cutting is usually explained on the basis of chemical gradients which are experimentally demonstrable. The organogenetically active substances hypothesized by Bouillienne or Went were deduced from studies of correlative phenomena which required the transport of unidentified substances from one part of the plant to another. Identification of such substances would be simplified if the experimental system permitted direct application of test substances to target cells without the intervention of a transport step. This method would not direct information on the integrated control of organogenesis by the whole plant, but it might be useful in obtaining information at the intracellular level. The transition to this kind of system was effetively pioneered by Skoog in his use of callus cultures as a method of bioassay for substances involved in organ formation.

When cell cultures are subjected to experimental media, the results are generally measured in terms of the kind and number of organs appearing after a considerable delay, without regard to immediate biochemical and cytological effects of test substances or alterations in the medium. Few reports of organogenesis in cell culture fail to conform to the same unrewarding generalization: establishment of the primary culture is followed, after one or more subcultures during which unknown cytological and physiological changes are occurring, by the production of roots on relatively high auxin levels and of shoots on relatively low auxin levels with or without a cytokinin. No adequate experimental system exists at present in which it is not only possible to control the time and place of initiation of organ primordia but also to isolate them for biochemical and ultrastructural studies during successive stages of development. Organ formation proceeding from extremely small clumps in suspension or plate cultures is a step in this direction.

Organ Initiation

Elucidation of the factors controlling organogenesis at the intracellular level will require knowledge of the metabolism peculiar to eumeristematic cells. A limited amount of information is available from biochemical analyses and from studies of the growth requirements of excised apices. The formation of eumeristematic growth centers in callus occurs when parenchymatous cells undergo a shift in metabolism to the "mitosis-determined" condition. This term was devised by Stebbins to describe the situation in which the relative rates of cell division and enlargement are such that successive generations of cells become progressively smaller until the eumeristematic condition is reached. This terminology is useful since it describes what happens at the gross cytological level during organ initiation without implying anything about the causal molecular events. The opinion of Haber and Foard that the extent and orientation of cell division do not play an essential role in the initiation or subsequent polarized growth of organs is not at issue here. In cell cultures, potential organ-producing cells are usually not identifiable and are therefore not susceptible to study prior to the eumeristematic stage.

Hypotheses with regard to the origin of growth centers are scarce. Torrey suggested that all organized structures in callus have a common origin in "activated" single cells which he called "meristemoids," a term devised by Bunning. Bunning long ago pointed out an impressive number of cases where the critical feature preceding the appearance of a specialized cell (*e.g.*, trichoblasts, guard cells, sclereids) was unequal division of a polarized cell. Of the two daughter cells, the one with the denser cytoplasm was termed a "meristemoid" since it had increased capacity for further cell division or for developing into a specialized cell.

Whether or not meristemoids, *sensu* Bunning, are involved in the origin of all organized structures in callus is unknown. There does seem, however, to be a fundamental distinction in the cytological events giving rise to organ primodia and vascularized nodules, since they may be separated experimentally in several ways. For example, habituated or senescent cultures may be incapable of organ formation but will continue to form growth centers containing vascular tissue. Although organs may originate in association with the cambia of vascularized nodules they also arise directly by dedifferentiation of parenchymatous cells unassociated with vascular tissue. Murashige found that exposure of tobacco callus to gibberellins prevented organogenesis without interfering with the development of vascularized nodules. It sense possible that gibberellin promoted vasculorization of growth centers which might otherwise have become organ primordia, since gibberellins have been shown to promote vascularization in a variety of experimental systems. The inhibition of organ formation in crown gall turgor was suggested by Kupila to be caused by this mechanism. However, Thorpe and Murashige on the basis of histological studies, suggested that gibberellins prevented the development of bud primordia in tobacco callus by causing breakdown of the starch specifically associated with prospective but-forming cells. Since intermediate cytokinin/auxin ratios also prevent organogenesis in tobacco callus, it would be of interest to have comparable data on the status or even recognizability of prospective organ-forming cells in such cultures. Although excellent histological studies of organ formation in cultured tobacco stem segments were carried out 20 years ago by Sterling, the equivalent studies do not seem to have been made for organogenesis in subcultured tobacco callus. Other cases of gibberellin inhibition of organ formation are known. On the basis of the fact that pre-existent primordia were not inhibited by applications of gibbarellic acid, Brian *et al.*, hypothesized that gibberellins prevented the initial divisions leading to organization of root primordia in pea and bean stem cuttings. A similar mechanism might be operating in the gibberellin-induced inhibition of floral initiation, since floral development is not prevented if the gibberellin is applied to the apex after the floral stimulus has arrived. It appears that this hormote constitutes a potentially valuable tool in studying the initial unique steps of organ formation.

Determination of Organ Type

When a particular treatment induces *de novo* formation of organs, root and bud primordia may be initially indistinguishable. The question naturally arises as to when organ type is determined. Morphogenetic plasticity of organ primordia has been demonstrated in both Pteridophytes and Angiosperms. Surgical experiments on fern apices demonstrated that young leaf primordia could be converted to buds. Similar experiments on Angiosperm apices were unsuccessful. Apparently, in Angiosperms a critical feature of the initiation of determinate organs is irreversibility as to organ type. Essentially similar conclusions may be drawn from Cusick's demonstration that developing floral primordia pass through a series of physiological states which are irreversible. There is evidence, however, that indeterminate primordia in Angiosperms go through a period of morphogenetic plasticity. Bonnett and Torrey found that young bud and root primordia in cultured *Convolvulus* roots were histologically indistinguishable. Appropriate culture conditions made it possible to obtain buds at presumptive root sites. They proposed that "these responses may be due to the initiation of a large number of primordia by auxin, which can then develop into either buds or roots depending on subsequent auxin levels." The proposal of a two-step formation of organs with the first step producing a generalized primordium and the second step determining organ type is not a new notion, since similar ideas can be traced back to Sachs Cutter proposed that two separate sets of factors might control organogensis: "phyllotactic factors" which determine the site of organ initiation, and "organogenic factors" which determine organ type, since in *Nymphaea*, identical sites in the genetic spiral may be occupied by leaves, vegetative buds, or flower buds.

To relate the information from *Convolvulus* or *Nymphaea* studies, one need only assume that the two sete of factors proposed by Cutter are qualitatively identical (*i.e.*, some chemical species) but quantitatively unlike. This assumption is fundamentally similar to the conclusions reached by Skoog and Miller on the basis of extensive studies of organogenesis in to bacco cultures. The same hormones required to initiate and maintain mitosis and cell division in excised tobacco pith—indoleacetic acid and a cytokinin—are also involved in the initiation and determination of organ types. (although there is an extensive interaction of hormones with phosphates and other substances, phenolic compounds in particular. The relationship between cytokinins and budding, discovered originally with tobacco callus, has since been observed in other experimental systems involving both seed plants and lower plants. The main thrust of Skoog and Miller's argument was that "quantitative interactions" among a common set of factors, and not unique morphogens, were involved in the regulation of "all types of growth investigated from cell enlargement to organ formation." Support for this position may be found in Hagen's studies of the regulation of growth and organogenesis in tobacco hybrid tumors by the nitrogen/hormone ratio. The dual role played by hormones and metabolities in regulating both cell division and organ type in *Nicotiana* callus is essentially similar to the dual role of such substances in regulating cell division and vascular tissue type in *Syringa* or *Phaseouls* callus.

Embryogenesis

Reports of the regeneration of whole plants in cell cultures have been numerous during the past 5 years. A variety of structures ranging from well-formed dicotyledonous embryos to obvious clumps of callus bearing fact, buds and roots to poorly defined forms of uncertain affinity have been reported under a bewildering variety of names. The suggestion has been made that any asexually produced structure, *in vivo* or *in vitro*, be called an "embryoid," reserving the term embryo for sexually produced structures. This proposal, in addition to relegating to second-class citizenship all of a which yield perfectly good embryos apomictically, overlooks the crurial point that the problem is not one of terminology but of technique. It is relevant, for example, what name is applied to the various kinds of embryonic propagules functioning in the normal life cycle of a species (*viz.*, vilar embryos in *Kalunchoe*, bulbils in *Lilium*, protocorms in orchids) since but are understood and recognized in terms of their distinctive ontogeny and final form. The only forms which are difficult to categorize by any means are teratological ones, and it is in this category that most of the plants formed *in vitro* must be placed. Many such structures were discovered in callus or suspensions after the culture had been through a successful of transfers on several kinds of media. often complex, during which time the developing organs were subjected to the deforming effects of high levels of growth substances. In the absence of a thorough study of organ ontogeny, including histological details, under the optimal circumstances for forming such organs, it is impossible to assess the significance of the results obtained.

The current need is to winnow this mass of data for the essential points regarding which cell types of the mature plant show embryological competence, the necessary chemical and physical factors involved, and the ontogeny of regenerated plants.

Origin of Embryologically Competent Cells

Few cases have been reported in which the specific cells of the explant giving rise to embryos can be unequivocally identified. Embryos have been reported in higher plant cultures started from excised seed embryos hypocotyls or stems storage root phloem or xylem, leaf mesophyll floral buds nucellar tissue etc. This is not intended to be a complete list, but it suffices to indicate the range of organs and tissues in a wide variety of Angiosperm species, including monocots, which can yield embryos. In addition, both haploid and diploid embryos have been obtained from several different cultured tissues of Cycads.

Perhaps the best identification of embryo origin from a specific cell type involves the epidermal cells of *Ranunculus sceleratus* and *Daucus carota*. However, the most interesting reports are those which claim the origin of haploid embryos from pollen grains. This phenomenon has been reported for *Datura innexia* and *Nicotiana tabacum*. It has been known for many years that developing microspores had the remarkable ability to yield embryo sac-like forms. It is apparent, therefore, that microspores may yield the male gametophyte, female gametophyte, or sporophyte. The ready interconvertibility of Pteridophyte generations thus has its counterpart in the Angiosperms. In *Hyacinth*, embryo sac-like forms were apparently unable to develop from microspores after the generative nucleus had differentiated. However, Guha and Maheshwari reported that in *Datura*, mature pollen grains with thick walls underwent internal proliferation to produce the embryo. It was apparently not known whether one or both nuclei of the pollen grain participated. In *Nicotiana*, the embryos were derived from microspores at the tetrad stage.

The ability of cells derived from so many tissues and organs to produce embryos is often taken as evidence for the generalization that differentiation of plant cells does not involve loss or permanent inactivation of genetic material. However, tissues of the mature plant contain large numbers of relatively simple parenchymatous cells which contribute to a developing callus and which may be largely responsible for the production of embryos. Final conclusions cannot be drawn about the genetic status of the full spectrum of mature cell types.

Ontogeny of Regenerated Plants

Three basic variations are found, or are reputed to be found, in the manner in which roots and shoots come into association with each other to form a plant. In one method, the regeneration of buds in callus is followed by formation of adventitious roots on the stem as the bud develops or the regeneration of roots in callus is followed by the formation of adventitious buds on the root. These cases are of no significance for embryogenesis. Secondly, independent regeneration of roots and buds in callus is followed by their integration into one axis. This phenomenon has been assumed to occur on a number of occasions, but there are no histological studies showing the intermediate stages involving union of the two organs. In any event, use of the term "embryoid" to describe clumps bearing only roots is pointless. Use of the same term to describe callus bearing both roots and leaves is equally meaningless unless the organs have been shown to be integrated and capable of functioning as an embryonic plant.

The third mode of whole plant formation is embryogenesis, i.e., formation of an integrated root-shoot axis by stages resembling the ontogeney of seed embryos. Review of this phenomenon necessarily involves a brief trip through the history of carrot cell culture. Nobecourt in 1946 reported the appearance of "de minuscules tiges portant chacune deux petites feuilles ovales, opposees, resemblant a de petits cotyledons" at three different places on a root which had grown from callus. The shoots appeared only in "cultures agees." Levine a few years later, observed roots in carrot callus grown on simple media containing auxin. Transfer of the callus to media lacking auxin resulted in the appearance of plantlets which bore "primitive" or cotyledon-like leaves. In the late 1950's, studies by Steward and his co-workers and Reinert again led to the discovery of entire plants in carrot cultures but differing ontogenetic sequences were proposed. Steward *et. al.*, observed the regeneration of roots from cambium-like cells in clumps growing in liquid media. The entire plants which subsequently appeared were assumed to have arisen by formation of a bud at a point on the clump opposite the root. Reinert's work with carrot callus led him to state that the shoots which were formed in his cultures were "fast ausschlieslich aus Adventivembryonen." Interpretation of his data is difficult because of the complexity and sequence of several kinds of media used, the extended time interval betweeh transfers and the appearance of new morphological forms (3 to 5 months) and the grossly deformed nature of the organs found. Similar difficulties beset the carrot culture system reported by Butenko and her co-workers in Russia. Although claims were made by all these workers that various components of the media were significant in including formation of organs, the only observation which seemed consistent was that roots appeared on auxin containing media and that lowering of the auxin content in the culture was essential to subsequent appearance of shoots. It should be noted that although these shoots were frequently assumed to have been derived from buds, histological studies demonstrating an unequivocal bud in carrot callus have never been published. The significance of this fact is uncertain, unless it indicates that such cultures never form buds and that all shoot systems are derived from embryos.

In any case, subsequent studies in a number of laboratories provided adequate documentation for the formation of normal dicotyledonous embryos in carrot callus and suspension cultures. Hypothetical ontogenetic sequences were proposed in which putative single cell progenitors gave rise directly to globular proembryos or to both filamentous and globular stages. It was found that continued exposure of proembryos to high auxin levels did not inhibit growth but caused them to proliferate randomly, forming disorganized starch-filled "proembryogenic masses" which could be carried through numerous subcultures Wetherell and Koukkari found these stable proembryogenic cells to have an extremely high phytochrome content. The heavy concentration of starch is also of interest, since the same phenomenon has been reported for nucellar embryo initials in some species and bud-forming cells in tobacco callus. In the case of carrot proembryogenic clumps, when the auxin level is lowered by transfer to auxin-free media, cells at the periphery of the clump utilize their starch and divide rapidly to form one or more embryos per clump. The development of multiple embroys in this fashion resembles eleavage polyembryony and is similar to the manner in which adventive embryos develop from experimentally damaged zygotic embryos of *Eranthis hiemalis*. In carrot suspension cultures, the proembryogenic clumps small enough to pass a 45μ sieve gave rise to normal dicotyledonous embryos within 10 days on auxin-free media a fact which emphasizes the difficulty in interpreting experiments where a period of weeks or months intervenes between transfer of callus to auxin-free media and the appearance of embryos, The possible failure of single cells to produce embryos in sieved suspension cultures was not assigned any significance beyond the well-established fact that few single cells divide in suspension cultures, a phenomenon which may be due to excessive leakage of metabolities into the medium or to the need for attachment to a solid substratum (another cell?), as demonstrated by Joshi and Ball.

Specific Factors Inducing Embryogenesis

Since readily expressed totipotency is a conspicuousfeature of at least some higher plant cells, there seems to be no theoretical obstacle to the developmental of experimental methods similar to those used in studying embryogenesis of the sea urchin or *Fucus*. The present obstacles are practical ones—namely, our inability to isolate a homogeneous population of embryologically competent cells and to establish a starting point through the introduction into the culture of some factor or factors which approximate fertilization. In the most extensive studies to date of the metabolism of carrot callus in relation to organ formation, Syono found that the callus passed through four distinct physiological phases which were characterized by formation of different kinds of organs and different responses to test media. Here again, however, detection of the organs depended upon transfer of the callus to auxin-free media, a procedure which permits further development of inhibited premordia but yields no information as to when or how they were induced. The inducing factors in embryogenesis are still not known, although several hypotheses have been advanced.

The crucial issues were first identified by Steward in 1958 (180) in a hypothesis which needs little description here. It consisted essentially of the proposal that exposure of cultured cells to coconut milk was a situation similar to the exposure of the fertilized egg to the endosperm and had, therefore, the same morphogenetic results. This hypothesis was of considerable heuristic value, although subsequent experimental work proved it to be erroneous. The embryogenic role of endosperm in the ovule, a necessary corollary of Steward's hypothesis, is not on sound theoretical ground either. The question remains: what substances, if any, induce cultured cells to behave like zygotes?

In considering this question, it is well to keep in mind that the entire ovule is peculiary embryogenic in nature, since cells of the nucellus and integuments commonly serve as embryo initials either in place of the egg or in competition with it in some taxa. Other tissues of the carpel do not behave in this way, pointing up the fact that information on the unique physiology of ovules is badly needed.

Recent studies with carrot cultures have emphasized the inorganic components of the medium. Halperin and Wetherell obtained data indicating that ammonium ions played a role in the induction of embryogenesis in wild carrot cultures. Later work indicated that the requirement was not absolute, since embryos were induced on other fowns of reduced nitrogen but leaf fewer in number than in ammonium-grown controls. It was proposed that ammonium ions might have developmentally significant effects not related to nitrogen metabolism. This hypothesis was consistent with the subsequent finding that cations or high salt concentrations were effective in inducing embryogenesis. A cation or salt effect is interesting in view of the demonstration by Braun and Wood that ions may be involved in activation of hormone biosynthetic systems. The role of reduced nitrogen remains unclear at present. Kato and Takeuchi found that yeast extract was as effective as ammonium in indcuing embryo development from cultured hypocotyl segments of carrot. Linser and Neumann found that reduced nitrogen was essential to carrot embryogenesis, but concluded that nitrogen and hormones influenced morphogenesis only indirectly through their effect on the rate of cell division. Reinert and his co-workers confirmed that ammonium was more efficient than other nitrogen sources in inducing carrot embryogenesis, but concluded that reduced nitrogen was not essential since cultures grown on nitrate alone would produce embryos. Unfortunately, none of these results from different laboratories are readily comparable, since they involved different varieties and organs of carrot, different basic media, different culture conditions, and inadequate quantitative methods of measuring embryogenesis.

The remaining factor which has been cited as playing a casual role in embryogenesis is that of cell isolation. Steward. in particular, has emphasized that isolation of single cells from the controlling influence of other cells, via the breaking of plasmodesmata, is a *sine qua non* for the expression of totipotency. Cases where the breaking of intercellular connections causes a dramatic change in growth pattern are well known. particularly in filamentous algae and fern gametophytes. However, embryos in subcultured callus or suspension cultures are usually derived from proembryogenic masses whose origin in the explant is unknown. In cases where embryos deveop directly on the primary explant, histological studies may reveal an extremely tenuous relationship between the embryo and subjacent tissues, indicating a possible single cell origin, or the embryos may be attached by a broad band of cells to the subjacent tissue, giving the impression that more than one initiating cell was involved. Definitive studies have not been performed, and it remains uncertain, whether the induction of embryogenesis in primary explants necessarily involves physical isolation (breaking of plasmodesmata) of single initiating cells, or whether some other form of physiological isolation of one or more cells is sufficient. Electron microscope studies of the initiation of nucellar embryos might be enlightening in this respect. It would also be informative to compare at the electron microscope level the initial stages of embryo development from hypocotyl epidermal cells of carrot or *Ranunculus* with the initial stages of bud regeneration from hypocotyl epidermal cells of decapitated flax, *Linum usitatissimum*, or ceanberry, *Vaccinium macrocarpon*. One frequently finds the misleading statement that such buds are derived from single epidermal cells. Mitoses may be restricted initially to one cell and its derivatives, but cell divisions eventually spread to the adjacent tissues as a vascular connection is established. Embryos do not induce vascularization of subjacent tissue in the explant.

Loss of Organogenetic Ability

The putative loss of ability to form organs may be associated with other changes in the cell strain involving growth rate-friability, nutrient requirements, and chromosome constitution. Cultures which show an increased growth rate, increased friability, and growth in the absence of exogeneous hormones are referred to as "habituated". Cultures exhibiting a decline growth rate and decreased friability have been referred to as senescent. Habituation is a gradual process of tumorization, since habituated tissues grafted into normal plants cause varying degrees of tumor development and, like tumor cells, are autonomous with respect to the synthesis of growth-regulating hormones. Symptoms of tumorization frequently precede obvious chromosome abnormalities. It is significant, therefore, that cultures which show some of the symptoms of habituation or tumorisation but which are still in the diploid or tetraploid condition remain capable of producing organs. Even when the etiology of tumorization is clearly genetic but does not involve aneupoidy or high levels of polyploidy, as in certain *Nicotiana* hybrids, the tumors are quite capable of producing roots and buds. Conversely, habituated cultures which do show a predominance of aneuploid mitoses are incapable of organ formation. It appears that genomic imbalance due to aneuploidy or high levels of ploidy is one direct cause of the inability of some cultures to form organs, a conclusion also reached by Torrey and Murashige and Nakano on the basis of studies with non-habituated callus strains. The low polyploid condition is, however, not incompatible with organogenesis *in vitro*.

Other causes of a failure in organ formation undoubtedly exist. It has been suggested that derived strains loss the ability to synthesize specific factors required for organogenesis, although the existence of such substances has always been problematic in the absence of chemical indentification. There are numerous reports of species which never produce organs at any time in their culture history, but few have been subjected to extensive analysis. Doering and Ahuja reported, for example, that callus cultures derived from both tumorous and nontumorous segregants of *Lycopersicon* hybrids fail to produce organs, even when transferred to media lacking auxins or other growth regulators which might suppress organogenesis. It would be of value to have detailed information on the chromosome constitution and histology of both explants and derived culture of such species. Some cell strains have been reported to remain diploid for years but the relationship of this fact to organogenetic potential was not made clear. Stable nonmutational change occur during aging of plants which show distinct phase changes during their life cycle. Cuttings from these plants during their adult phase show a decreased capacity for regeneration. Similar nonmutational changes may occur *in vitro*.

5

Ecological Anatomy of Various Plants

Alpine climbers pausing to rest on rocky outcrops in the midst of widespread icefields have noticed, upon close examination, that plants (lichens) are growing upon the seemingly barren rocks. In Death Valley, Calif., the hottest and driest region of this country, there are more than three hundred species of native plants. That plants are found growing in snow, in the waters of the Great Salt Lake, in hot springs, on beach and desert sands, and even underground in caves and mines attests to the wide range of their adaptability.

These rather extreme examples illustrate the fact that plants exist in almost every known environment. The adaptations and relationships of plants within less extreme environments may be just as striking and significant as those just mentioned, The scientific study of the relationship between plants and their environment is known as *ecology*, or, more strictly speaking, plant ecology, since the same types of relationships also exist for animals. The study of plant ecology merges into plant geography, which treats of the distribution of plants over wide arers and attempts to account for the distribution and migration of species.

Plants usually live not alone but in *communities* composed of one or more species living in a specific area under similar conditions and affecting one another. Such communities may be viewed as ecological units. A community may consist of a mass of algae floating on a pond, lichens on a rock, a field of corn, an old pasture, a marsh or bog, the weeds of a vacant city lot, an oak-hickory forest, or even larger units. The study of how plants (and animals as well) in a community live together constitutes an important part of the field of ecology.

Closely related to the concept of environment is the *habitat*. This term is applied to the place where a particular kind of plant or community of plants usually llves. A habitat may be a south-facing, warm, and dry slope; a mud flat: a damp ravine; a marsh; the floor of a moist woodland; or a sandy beach. It implies a particular group of environmental features and is therefore more specific in meaning than environment as a whole.

The growth and reproduction of plants is intimately connected with such environmental features as water, light, physical and chemical aspects of the soil, wind, and temperature. The great variations in these and other factors from region to region are in large part responsible for the differences among natural environments and for the kinds of plants found in them. The ecological factors responsible for these differences act as a whole, producing, as a result of their combined effects, a characteristic environment. Within this framework the contribution of each factor is fairly distinct, although its effect may vary over a comparatively wide range. It is possible to look at ecology in two ways; one may consider the ecological factors and their effects on habitats, and, one may consider habitats in terms of their typical plant life and characteristics. Both these points of view are essential and are included in this chapter.

Ecological factors. Ecological factors are customarily classified as follows:

(1) *climatic* factors, such as precipitation, length of day,

atmospheric humidity, temperature, wind, and intensity of light;

(2) *soil* factors—the chemical and physical nature of the soil, the soil water, the soil temperature, and the soil atmosphere;

(3) *biotic* factors, those resulting from the activities of plants themselves and of man and other animals.

These categories overlap in considerable degree. The amount of rainfall affects the water content of the soil, and both precipitation and temperature modify the chemical and physical nature of the soil. The intensity and duration of sunlight may be determined by the climate or by a biotic factor, such as shading by other plants. Moreover, plants are influenced by a host of agents, not by one factor alone. The effects of all must be integrated in order to obtain a comprehensive picture of the plant in relation to external infiuences. That the forests of Ohio are deciduous rather than coniferous is a result of climatic conditions, past and present. It is easy to observe mayflowers, trillium and Solomon's s-seal growing in open woods, and fleabanes, asters, and thistles growing in adjacent open fields. But an understanding of the mechanisms which have segregated these plants into their special habitats is more difficult.

Ecological studies thus become extremely complex. But in spite of the difficulties, the study of plants in their environments has yielded a large body of ecological facts, contributing both to a better knowledge of plants themselves and to a more effective use of plant, soil, and water resources. Every farmer or gardener is a practicing ecologist, since by such practices a^s^ cultivation, fertilization, irrigation, and spraying he affects plant behaviour by changing the environment of the plant. The farmer who incorporates the best practices of soil conservation and agriculture in his work discovers that he is creating a specialized environment. In it, highly specialized, cultivated plants will produce a high crop yield, while maintaining or even improving the soil. Such an environment may positively affect the farmer's livelihood.

Climatic Factors

Temperatur·. Temperature and precipitation are the chief climatic factors which determine the occurrence and range of various plant species and the pattern of vegetation. They are the factors most closely related to plant survival. Temperature influences all plant activities—the absorption of water, photosynthesis, transpiration, respiration, germination, growth, and reproduction. Temperature extremes are important, but significant also are their duration and the length of the growing season, or frost-free period. The latter is an important factor in determining the areas in which crop plants can be grown.

One factor that restricts cotton growing to the South is that this plant requires a growing period of at least 200 days. The northern limit of profitable production is also determined by an average summer temperature of 77°F. The long growing period in the Imperial Valley of California, in the lower Rio Grande valley of Texas, and in Florida makes it possible for these regions to supply the North with out-of-season vegetables. Several crops a year are possible in these regions. On the other hand, some varieties of corn and spring wheat will mature in less than 100 growing days. Potatoes. cabbage, peas and other garden vegetables mature in a growing season of 75 to 90 days. Large areas of Alaska have a growing period of more than 90 days, in addition to other factors that make farming possible.

Low temperatures may affect plants both during the dormant period and in the stage of active growth. Winter injury may result in the death of the plant or in damage to the roots, bark, and buds. Many crop plants, both woody and herbaceous, are injured by frost in late spring. Damage to citrus, peach, and other crops is often reckoned in millions of dollars. Injury and death of plants at high temperatures is most commonly the result of a high rate of transpiration accompanied by a deficiency of water in the soil.

Water. Because precipitation directly affects the amount of available soil moisture, the annual rainfall is a major factor in determining the distribution of plants. But its influence may be modified by the distribution throughout the year, the amount falling at any one time. the slope of the land. the permeability and water-holding capacity of the soil, the wind velocity, and the temperature. In general, forests occupy areas where the rainfall is highest; deserts occur where it is lowest; areas of intermediate rainfall are grasslands.

The seasonal distribution of rainfall, as mentioned above, may be more important than the total amount. For agriculture, spring and early summer rains are generally preferable to those of fall and winter. If rainfall is uniform throughout the the year, forests may exist in regions of relatively low total rainfall. High temperatures, which increase the rate of evaporation from the soil and the rate of transpiration, are perhaps the most important factors influencing the effectiveness of rainfall. In long-range agricultural planning, it is not enough to know the annual rainfall and its seasonal distribution. The rate of evaporation and degree of retention in the soil must be known so that the moisture actually available to crops can be calculated. Light rains in hot, dry weather will usually have no effect upon the soil moisture content, for the water quickly evaporates from the soil surface. Heavy rains of short duration may also have little effect upon soil moisture, for the run-off may be great.

Water in relation to habitat and plant structuro. On the basis of the nature of the habitat, plants may be classed as (1) *mesophytes*, (2) *xerophytes*, and (3) *hydrophytes*. Mesophytes grow in an environment which is neither very wet nor very dry, such as the meadows and forests of the temperate zones and corresponding regions of the tropics. Because most vascular plants are mesophytes, their structures and adaptations are already familiar to us. There is no hard and fast division between these plants and those adapted to drier environments on the one hand and to wetter ones on the other.

Broadly defined, a xerophyte may be regarded as a plant adapted to survival under conditions of a deficiency of water in its habitat. This deficiency may be persistent or periodic. Xerophytes are characteristic of desert and semidesert regions. They also occur in mesophytic regions where available water is occasionally deficient, as on sand hills, beaches, rocky ledges, or cliffs. Even where the annual precipitation is sufficient to permit a mesophytic vegetation, xerophytes will be found in place of mesophytes if the climate is characterized by sharply defined wet and dry seasons. The xerophytes are better able to withstand the seasonal droughts. Still other kinds of xerophytic habitats, to be discussed later, are found.

Hydrophytes grow in or near the water, partly or entirely submersed. Some grow on land with their roots in saturated soil. Both xerophytes and hydrophytes probably originated from mesophytes and by evolutionary change have become adapted to new environments. In the course of this evolution many changes in physiology, structure, and behaviour related to the environment have arisen.

Form and structure in xerophytes. The xerophytes are a very diverse group and possess no anatomical or physiological features in common. Xerophytes may be classed as (1) succulents and (2) nonsucculents, sometimes termed "true" xerophytes.

The succulents are regarded as highly specialized xerophytes. Among the plant families containing succulents are the cactus, spurge, milkweed, stone-crop, lily, and amaryllis. The chief characteristic of the succulent type is that the bulk of the plant bodyis composed of water-storage cells which supply the plant during periods of drought, when water cannot be obtained from the soil. The succulent organs are generally the stems or leaves, rarely the roots. If the leaves are succulent, the stem is generally much reduced: if the succulent organ is the stem, the leaves are reduced or absent. The volume of the shoot is great in proportion to the surface exposed, and this, combined with a well-developed cuticle and other features, retards the rate of water loss so that the transpiration rate is low. The succulents are said to resist, rather than endure, drought.

Succulents illustrate clearly the principle that unrelated plants, under similar environmental conditions, commonly develop striking similarities in external from. For example, species of the spurge family growing in the deserts of Africa and Australia show such marked resemblance to some of the cacti of the American deserts that in the absence of the flower they might be considered close relatives.

Most of the nonsucculent xerophytes, like the succulent types, are perennials, and include herbaceous forms, mostly grasses, as well as woody species. Many of the latter are more or less evergreen, or at least retain their leaves during periods of drought. The leaves of such plants exhibit *xeromorphic* modifications which enable them to survive during periods of transpiration stress—that is, when the rate of water loss tends to be greater than the rate of water intake by the roots. Among xeromorphic modifications (Fig. 5.1) are (1) small, thick, leathery leaves, (2) a dense layer of hairs on one or both surfaces of the leaf, (3) a thick cuticle, (4) small and closely compacted leaf cells, with thickened cell walls, (5) well-developed mechanical tissues, with numerous vascular bundles, (6) capacity for folding or curling of the leaf (Fig. 5.2), (7) well-developed palisade tissues and (8) sunken stomates. The capacity for folding os curling is relatively common in certain xerophytic grasses. The stomates are located chiefly on the upper surface, and as the leaves curl or roll upward, the rate of water loss decreases.

It was long assumed that the xeromorphic modifications of the leaves of evergreen xerophytes were usefulin reducing the rate of stomatal transpiration. Experiments show, on the contrary, that per unit of leaf area the transpiration rate of

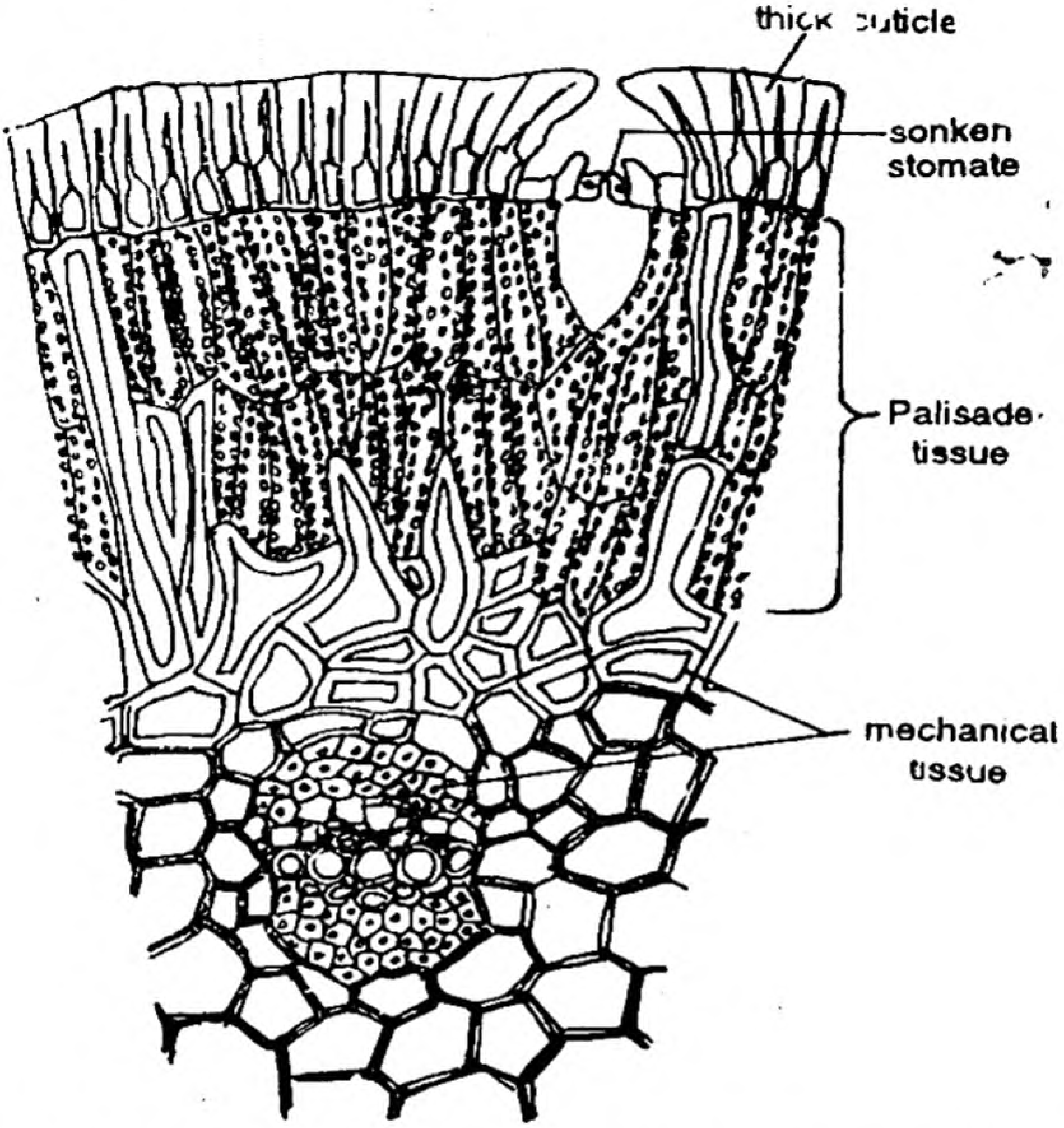

FIG. 5.1: Portion of cross section of a leaf of Hakea, an Australian xerophyte.

such plants, when the stomates are open and soil water is available, is commonly as great as or even greater than that of mesophytes under similar conditions. These structural features are of value, however, under conditions of drought, for, when the stomates close, the thick cuticle and in some degree the covering of dead hairs bring about a reduction in cuticular transpiration. The mechanical tissues prevent the collapse of the leaf, which would otherwise result from dehydration. The effect of desiccation upon such xerophytes is thus much less than upon a mesophyte. The former, under adverse conditions, are able to conserve water and keep its loss to a minimum.

The capacity of many xerophytes for survival probably lies not only in structural features but also in resistance of the protoplasm to heating and drying. There is evidence to show that the colloids of drought-resistant plants have a greater water-retaining capacity than those of mesophytes. This property may be of significance in reducing water loss and in prolonging the life of the plant during periods of water scarcity.

Numerous controversies have arisen over xerophytes and their relations to their habitats, and many aspects of the subject are still not understood. Although a dry habitat is commonly regarded as characteristic of xerophytes, xeromorphic modifications are found in plants growing in habitats not usually regarded as dry. The epiphytes (plants growing upon other plants) of the canopy of many tropical rain forests, for example, include succulents, such as cacti, and nonsucculents with xeromorphic modifications, such as thick, leathery leaves. Among these nonsucculents are heaths, orchids, and many other kinds of plants. Such epiphytes may be regarded as xerophytes, for they are dependent upon rainfall for their entire water supply and between rains must be able to endure or resist drought if they are to survive.

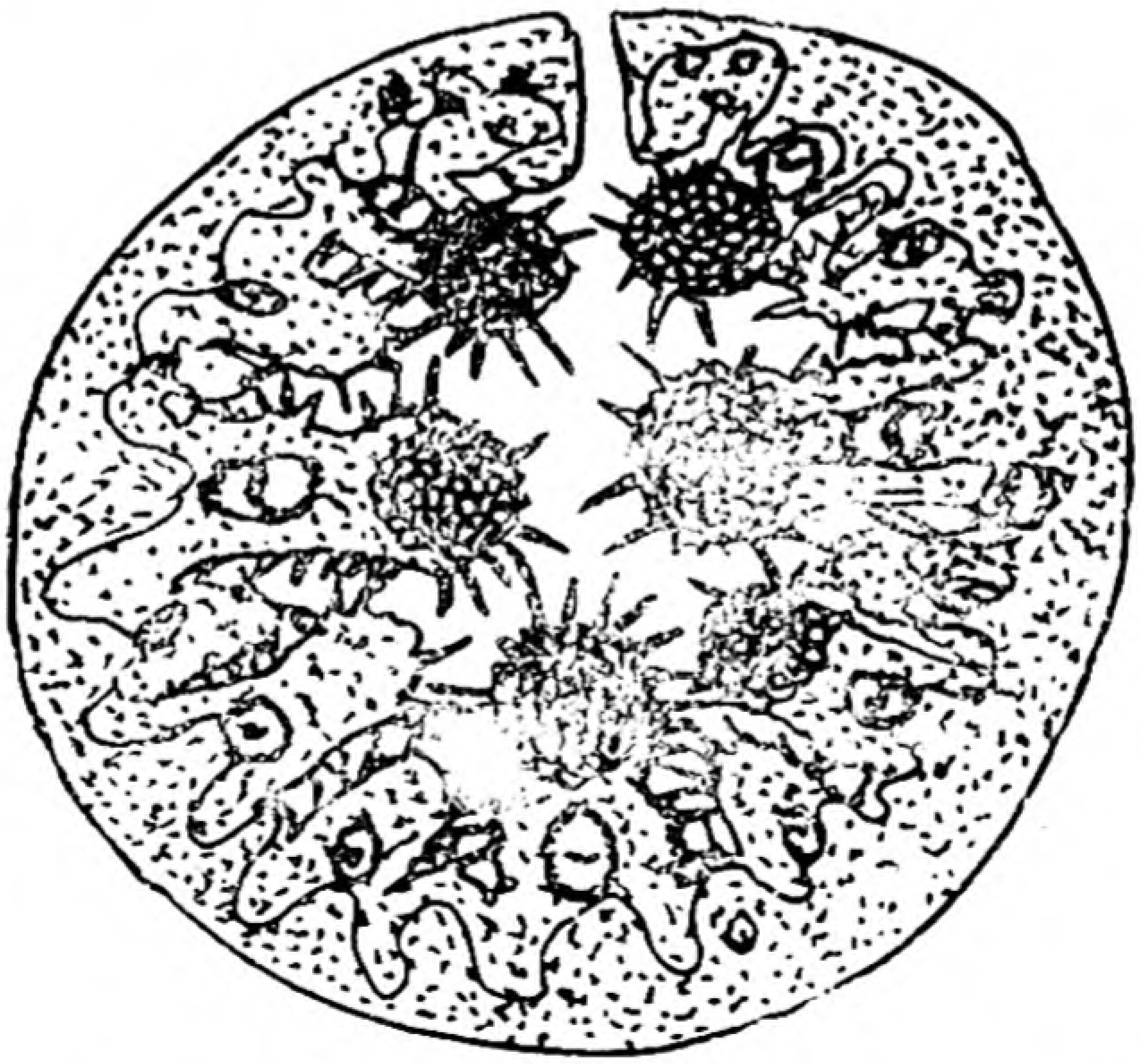

FIG. 5.2: Rolled leaf (cross section) of a xerophytic grass (Ammophila).

Xeromorphic modifications are found also in conifers and in evergreen flowering plants, especially those growing in alpine regions. Conifers may be subjected to drought when the soil is cold or frozen. Evergreen alpine plants are exposed to drying winds, periodic drought, and a cold or frozen soil from which water is obtained with difficulty.

All nonsucculent xerophytes do not possess xeromorphic modifications. The leaves of many desert shrubs are thin, and the transpiration rate is high when water is available. They may have extensive root systems which penetrate deeply into the soil. During periods of drought, the leaves of these plants become brown and shriveled or else the plants are deciduous. Like xerophytes with xeromorphic modifications, they endure drought during rainless periods.

Another group of nonsucculents which possess no such modifications are the desert ephemeral annuals, which carry on active growth only during a brief rainy season. When the rains come, the seeds germinate; the plants grow rapidly and produce flowers and seeds within a period of a month or six weeks. As drought sets in, only the seeds survive. Such plants evade drought because they are able to complete their life cycles within a brief period, before the soil dries. Since the ephemeral annuals probably encounter little difficulty in obtaining water during their period of growth, their classification among the xerophytes may be questioned.

Form and structure of hydrophytes. Some hydrophytes are free-floating plants; others grow submersed. Still others are amphibious. Such plants grow up into the air, but the roots or rhizomes are anchored in the mud, perhaps beneath a foot or so of shallow water. Internally, hydrophytes are commonly characterized by a well-developed aeration system (Fig. 5.3). This consists of greatly enlarged intercellular air chambers, which extend throughout the plant. Mechanical and conducting tissues are often reduced in amount, and their cell walls are thinner than usual. In submerged plants the cuticle may be absent from leaves and the roots reduced in size or absent. The

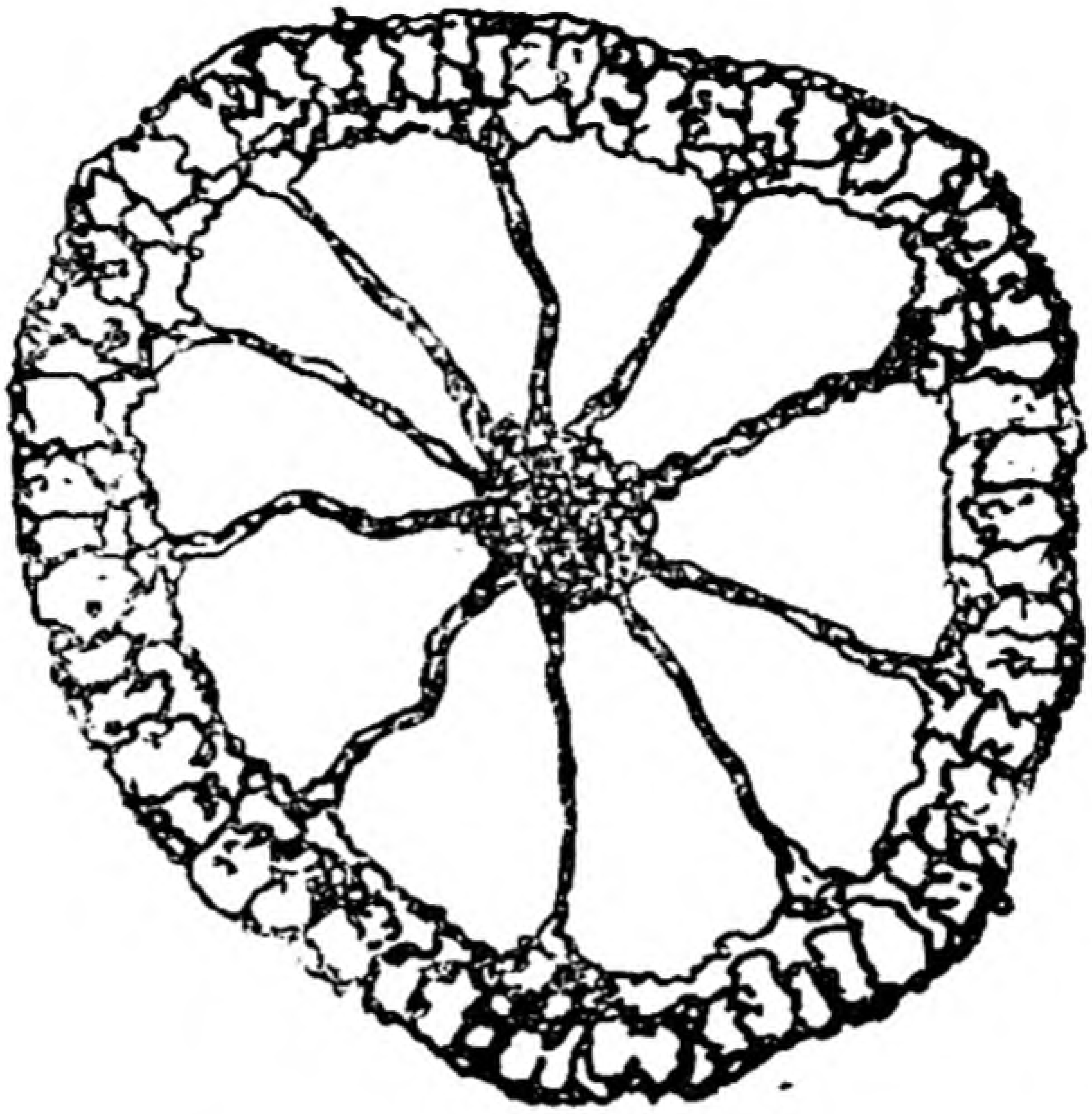

FIG. 5.3: Cross section of stem of a hydrophyte (Cerato-phyllum), showing air chambers and reduced vascular system.

leaves are commonly modified and may be thin, ribbonlike, or dissected. Some amphibious plants, such as cattails, although possessing specialized air chambers, have retained the well-developed mechanical and conducting tissues of their ancestors, particularly in their rhizomes.

Plants which grow entirely submersed do not have direct access to the oxygen of the air. The oxygen used in respiration comes either from their own photosynthesis or from the atmospheric oxygen dissolved in the water. Anaerobic respiration also occurs. The rhizomes, roots, and other submersed organs of partly submerged plants are in large degree independent of the oxygen in the surrounding water. Most such organs are able to carry on aerobic respiration because free oxygen diffuses downward from the aerial organs of the plant through the air passages. The oxygen may in part be derived directly from the air but is mostly the result of photosynthesis. Semi-submerged plants also respire anaerobically for considerable periods of time. Both aerobic and anaerobic respiration probably proceed simultaneously for much of the life of the plant.

Light. Light is the third important ecological factor which helps determine the distribution of plants and the formation of plant communities. The effect of light upon plant growth, flowering, and photosynthesis has already been discussed. Light is subject to cyclic fluctuations in intensity, depending upon the altitude, latitude, season, and climatic conditions. Locally, the intensity of light may vary considerably. However, the amount of light received per acre is relatively uniform over wide areas.

One obvious effect of low light intensity is the poor growth of grasses when shaded by trees with dense foliage. In the weak light of the forest floor, undergrowth may be entirely absent. When trees grow closely together. shade causes the death of the lower branches.

Many species have rather definite light requirements. Some species possess the capacity to survive and grow in light of low intensity, as on the floor of a forest. These are said to be *shade tolerant*. Other species, called *shade intolerant*, require a high light intensity, in many cases at least 50 per cent of full sunlight. These terms are usually applied to forest trees, but they may also be used with shrubs and herbs.

Tolerant and intolerant species may be identified by a study of young trees in the shade of a forest canopy. Certain woody plants, such as sugar maple, black gum, beech, basswood, hemlock, fir, and spruce, are shade tolerant. Other species, such as trembling aspen, the birches, willows, yellow popular, shagbark hickory, and some pines, are shade intolerant. Among tree species, the light requirement is chiefly important in the seedling stage.

Although tolerance is important in determining the establishment of a species in a given area, competition for water and for essential elements by roots may be more significant than is generally believed. In some areas light is the more important factor; in others, light and root competition operate together to influence the occurrence or predominance of a given species. A knowledge of the tolerance of the valuable timber trees is extremely important in the management of forests and woodlands.

THE SOIL REACTION

Extensive studies have shown that the reaction—acid or alkaline—of the soil is important for plants, although it constitutes only one of the factors affecting growth and distribution. The acidity or alkalinity of the soil is usually expressed in terms of *p*H. The *p*H of a solution is a measure of the degree of acidity or alkalinity. On the *p*H scale, which extends from 0 to 14, 7 is the point of neutrality. This is the *p*H of pure water. Values below 7 indicate acidity. A *p*H of 6.0 to 6.8 is slightly acid; a *p*h of 5.5 to 6.0 is designated as moderately acid; a *p*H of 5.0 of 5.5 is said to be medium acid; and below this range the soil is extremely acid. A *p*H above 7 indicates alkalinity. For practical purposes, a *p*H between 6.6 and 7.3 is considered neutral. Soils of grasslands and arid or semiarid soils are likely to be neutral or alkaline; those of areas with a moderate to heavy rainfall are usually acid. Many soils of the arid western Great Plains and intermountain regions, for example, are rich in lime as well as in other mineral nutrients.

Many wild and cultivated plants grow well in soils which vary over a wide *p*H range. Others are restricted to, or at least make better growth in, soils which are distinctly acid or distinctly alkaline. Among the plants which are especially sensitive to the effects of acidity or alkalinity are certain ferns, many orchids, and most legumes and heaths. Blue-berries, cranberries mountain laurel, and rhododendron are inefficient in the absorption of iron and grow well only in acid, or sour, soils. The distribution of a number of interesting wild species is limited to limestone region. Many crop plants flourish best at a *p*H of 6.0 to 7.0. If soil tests indicate a *p*H below 6.0, some crops will benefit from an application of lime. Crops with a distinct preference for slightly acid or slightly alkaline soils include peanuts, alfalfa, and sugar beets.

The relation of soil reaction to soil fertility and plant distribution is complex. Acidity affects plants indirectly through its influence on the physical condition of the soil, the availability of essential elements, and the activity of microorganisms.

Biotic Fators

Any study of the factors which affect plant must include a consideration of other living organisms, such as other plants, both green and nongreen, and all animals, including man. The activities of any of these may result in changes in the habitat and produce profound effects upon growth, structure, reproduction, and distribution of plants. The most important changes in the environment result from the competition of plants with one another.

Competition. As long as plants live as isolated individuals, growth is influenced only by such factors of the environment as climate and soil. But when plants live in groups, or communities, they compete with one another in all stages of development, from the seedling to maturity. This competition is primarily for water, for light, and for essential soil elements. The problem of actual space for the growth of roots or other underground parts is seldom a factor in competition.

Plants do not complete if the environment supplies essentials in excess of their requirements. When plants grow close together, however, roots invade the zones from which supplies of water and nutrients for other plants are obtained. The foliage of one plant tends to shade that of another. As a result of competition, certain more vigorous and better adapted plants become dominant. Others, less vigorous, are suppressed in greater or less degree. The invasion, or movement of individual plants or species into new environments already occupied, depends upon the ability of the invaders to compete with established species and individuals for the environmental essentials for growth.

The nature of the requirements for which plants compete varies in different habitats. In the forests, light is perhaps the most important, whereas in grasslands, deserts, and cultivated soils, competition is chiefly for water and minerals. Competion is a process of vital interest from the standpoint of food production. Most cultivated plants could not compete in the wild. Under cultivation, competition is, in large degree, under man's control. The destruction of weeds, for example, eliminates competition for water and light between them and crop plants. The proper spacing of seeds at the time of planting controls competition between plants of the same kind and results in greater yields. If too many seeds are planted in a given area, the yield may be reduced as a result of competition for an inadequate supply of water and nutrients. On the other hand, thin planting, with the aim of reducing competition, is not necessarily profitable, for the yield may fall short of the maximum because the evailable nutrients, water, and light have not been utilized fully. Competition should be permitted to operate to the extent to taking advantage of all the potentialities of the habitat.

The most striking effects of the competition for light are found in tropical rain forests other than in mesophytic forests of temperate climates. In regions of uniform temperatures, a long growing season, and precipitation of 60 or more inches evently distributed throughout the year, the luxuriance of plant growth is greater than in any other regions of the earth's surface. In such an environment plants are relieved in large measure from competition for water, and survival depends chiefly upon exposure to light adequate for growth.

Lianas and *epiphytes* are prominent adaptations which permit certain rain-forest plants to survive in the struggle for existence. Lianas are climbing perennial plants with woody stems which may vary from about 1/4 inch to more than a foot in diameter. Certain temperate-zone plants, such as clematis, grape, ivy, and honeysuckle, may also be termed lianas, but they are usually small compared to the lianas of the rain forest. Here, rooted in the soil and supported by the trees around them, lianas extend upward until their leaves form a part of the forest canopy, sometimes 200 or more feet above the ground. In this location, the leaves as well exposed to the sunlight.

Epiphytes, or "air" plants, grow upon other plants, usually trees. They are not parasitic but manufacture their own food. Although they live attached to other plants, they have no structural relationship with them. The support they obtain place them more favourably in relation to light. Many kinds of plants may be epiphytic. Among them are ferns, lichens, mosses, orchids, and members of the arum, pineapple, and cactus families. Epiphytes also grow on telephone poles and other tall object, as does the Spanish moss (*Tillandsia*), a rootless flowering plant of the pineapple family. This plant, abundant in parts of the South, absorbs rain water and dew through minute scales which cover the leaves and the greatly elongated selender stems. It obtains its essential elements from dust and rain water. Most epiphytes obtain their nutrients from decaying organic matter which collects around their roots.

Although epiphytes occur in regions where atmospheric humidity and rainfall are high, their roots, stems, and leaves are frequenly modified as water-accumulation organs. Some epiphytes are found in the lower levels of the forest; others grow in situations exposed to full sunlight. Lianas and epiphytes are plants which have varied in ways that favour survival in the struggle for light.

Plant Succession

Competition in a plant community usually beings about changes in the conditions affecting plant growth. Plants react with one another to change the habitat in relation to light and the supply of water and nutrients. Tall plants shade lower plants and bring about changes in the temperature of the air and soil, in relative humidity, and in force of the wind. A dry soil may become moist and more suitable for the growth of seedlings because humus accumulated by the decay of plant tissues has greater water-absorbing and to some extent greater water-retaining powers.

As the environment becomes modified, the kinds of plants which compose the plant population also undergo change. New species invade the area and, if these are better adapted to the changed environment, they become established and supplant the origial inhabitants. In the course of time a given territory will be occupied successively by different plant communities, a process known as *succession.*

As plants compete more and more with one another, certain species become *dominant*—that is, they largely control the environmental conditions upon which the growth of other plants depends. The course of a succession is marked by a sequence of dominant forms, for as new plants invade, the degree of competition increases until one kind of community is replaced by another. Eventually, the vegetation tends to become stabilized—that is, the kinds of plants occupying an area exert such an influence upon the environment that the rate of change diminishes to a minimum. The end of a succession, characterized by the general aspect of the vegetation or by the constituent species, is known as the *climax.* The great plant formations, such as forest, grassland, and, tundra, are climax formations. The dominant species of such climaxes, as the result of climate and other factors, are different from place to place, and form climax *associations* or subdivisions of the larger climax formations.

The western forests of this country, for example, are composed of such associations as western hemlock-western red cedar, spruce-fir, or western yellow pine-Douglas fir. Among the climax associations of the Central Hardwood forest (Fig. 5.4) are oak-hickory and beech-maple. Deciduous-coniferous associations are prominent in the Northern forest. The grasslands are composed of a number of associations, each characterized by two or more species. The climax associations of the tundra may be lichen-moss in some regions, dwarf shrubs in others, and a mixture of grasses, sedges, and herbs in still others.

Successions are of various kinds, but all are composed of a number of stages, each characterized by different forms of plant life. The first plants to become established in any succession constitute the *pioneer* stage. The nature of the pioneer plants is determined in large part by the nature of the soil or rock and by the water supply. Some successions, termed *primary*, begin in areas which previously have been unoccupied by plants, such as open water, bare rock, or sand. *Secondary* successions begin wherever the existing vegetation has been destroyed without denuding the area of soil. Secondary successions commonly develop after forest fires. lumbering operations, floods, and erosion. and on abandoned agricultural lands. Successions are so varied that only a few examples. illustrative of the general principle, can be described here. The first of these is a primary succession which begins on bare rock.

Primary succession. Succession on bare rock surfaces resulting from glaciation or from erosion by wind or ice commonly begin with lichens as the pioneer stage. These plants grow only when water is available, but in the intervals between rains they can remain alive even though desiccated. The mechanical and chemical action of the lichens on the underlying rock loosens particles, which, together with the decaying lichens, make up the first thin layer of soil. Simple crustlike lichens may be succeeded by larger, leafy forms, which grow on the slight accumulation of soil and humus.

Lichens are followed by mosses, which like lichens, are able to survive and reproduce in a xerophytic environment. The mosses shade the lichens and successfully compete with them for water and nutrients. The death and decay of the older mosses ofen produce a mat over the rock surface. As this mat becomes thinker and develops a greater water-holding capacity, a third stage in succession follows. This consists of annual and perennial herbs, including gasses and occasionally ferns. The soil is increased in thickness by disintegration of the rock and by the decay of the roots, stems, and leaves of such plants; more nutrients become available, and a fourth stage, dominated by shrubby plants, appears. The herbaceous plants of the preceding stage, now shaded, tend to disappear.

The shrubs, in turn, further enrich the soil and in other ways bring about conditions suitable for the development of the tree stage in the succession, and such forms constitute the climax in a forest climate. The first tree species to appear, however, may not be the climax species, but forms adapted to relatively dry conditions. As the soil moisture increases, such species will gradually be replaced by more mesophytic types. These may become dominant because their seedlings are shade-tolerant in contrast to the shade-intolerant seedling of the first tree invaders.

The species of the climax reproduce themselves, replacing the individuals that die, and remain the same unless the climate changes or the community is distured by fire, man, or other agencies. Succession on bare rock does not always follow exactly the sequence just described. If crevices accumulate small amouts of soil of if deposits of sand and gravel occur on the rock surface, the earlier stages in the succession may be by-passed, with herbs, shrubs, or even trees becoming established more rapidly.

Note that the succession described above leads from xerophytic to mesophytic types of vegetation. This situation is common in regions of adequate rainfall where primary successions begin on areas devoid of soil. Another example of such a succession is found on sand dunes, where drought-resistant grasses are commonly the pioneers, followed by shrubs and trees.

Primary succession on land may lead to a grass-land rather than to a forest climax, as evidenced by the grassland areas of North America and many other parts of the world. Three climax types have been recognized in North America: (1) the tall-grass prairie, (2) the mixed-gras prairie, and (3) the short-grass plains.

Primary successions also proceed in open bodies of water, such as ponds and lakes Fig. 5.4. The first step here may consist of submerged aquatic plants, which, accumulating after death and decay, gradually raise the lake bottom. Such plants grow most luxuriantly in shallow water, and the accretion of organic and mineral materials therefore proceeds more rapidly near the shore than in deeper water. As the bottom is raised, a second, or floating, stage follows, characterized by water lilies, broad-leaved pondweeds, and other plants. These plants are rooted in the mud, and their leaves float upon the surface, shading the plants below them. The death and decay of the plants of the floating stage contribute further to the soil-building process. With decreasing depth of water, a swamp stage is initiated. The area is invaded by amphibious plants, such as cattails, rushes, and shrubby hydrophytes. Eventually the soil becomes dry enough to afford a foothold for terrestrial species, such as grasses, sedges, and other low herbaceous plants. These are shaded by the succeeding shrub stage, which is followed in turn by a climax of trees.

Secondary succession. Secondary successions usually result from the activities of man and are, therefore the most common type of succession, especially in settled regions. They have fewer stages than primary successions, and the climax is reached more quickly.

The stages which follow the removal of the original vegetation vary widely in different regions, depending upon the climate, the soil, and the kinds of plants which invade the denuded area. Burned or lumbered lands may be colonized by grasses and other herbs, both annual and perennial. Fireweed is a typical example. These may be followed by shrubs, such as sumach, or by weed trees, such as aspen and wild cherry.

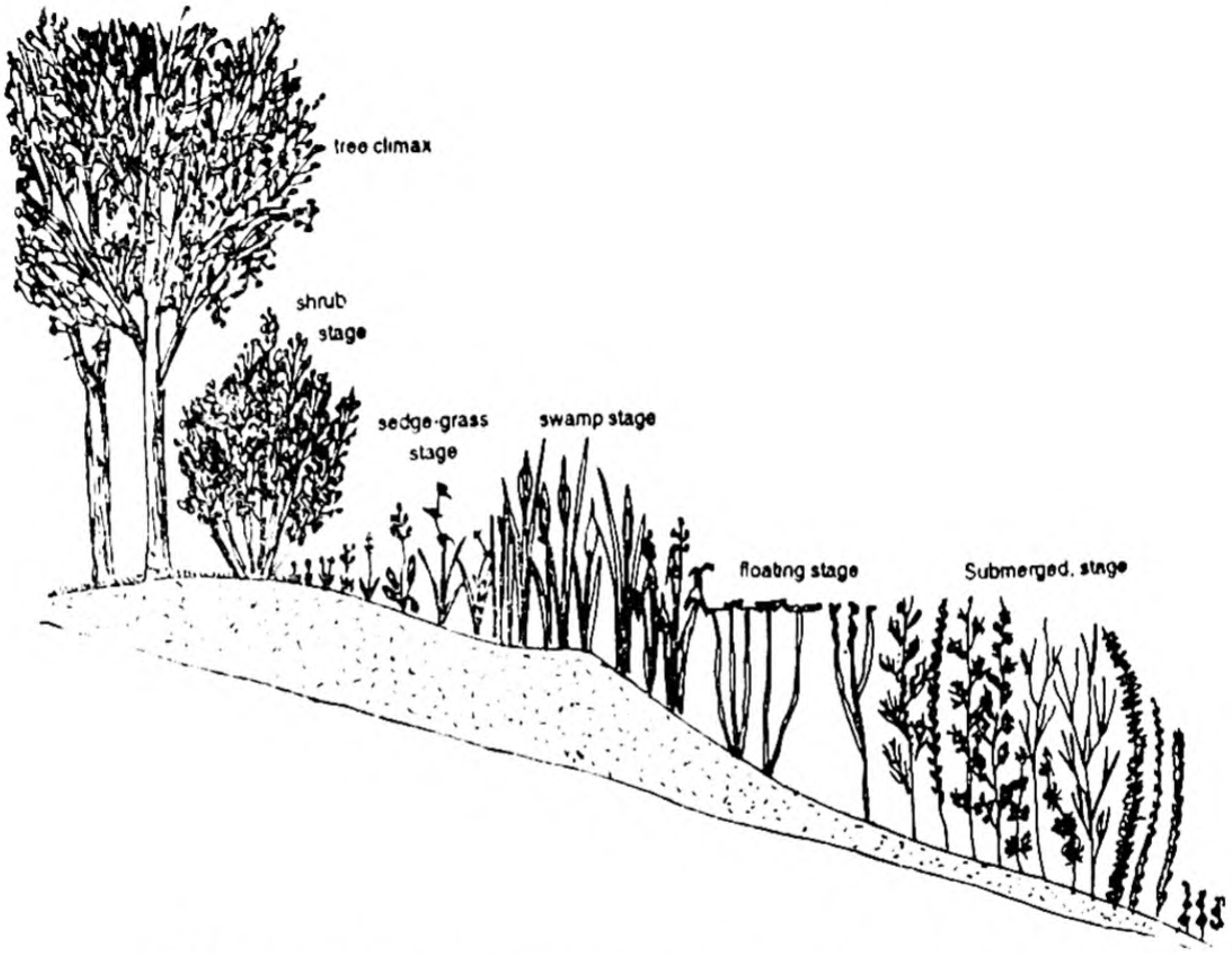
tree climax
shrub stage
sedge-grass stage
swamp stage
floating stage
Submerged. stage

FIG. 5.4: Diagram of a primary succession from water. The water surface ir shown in red.

All these have seeds that are easily disseminated. If undisturbed by man, the area will again be covered, after a period of time, by a deciduous or coniferous forest similar to the original climax.

Succession on abandoned agricultural land may proceed from herbaceous annual weeds to perennial weeds and grasses, then to prairie or woodland. A typical succession is revealed by studies of abandoned upland farms in the Piedmont area of North Carolina. Scattered stands of undisturbed hardwoods, including trees 200 to 300 years old, show that oaks and hickories dominated in the forests before the advent of the white man.

The early stages in the succession follow each other rapidly. In fields abandoned for one year, the most conspicuous species are the annual crabgrass and horseweed. In the second year the dominants are the common ragweed and a tall, bushy, white-flowered aster. By the third year, broomsedge (a grass) has formed massive clumps whose tops shade surrounding plants and whose extensive fibrous roots compete with the roots of smaller plants for water and minerals. The broomsedge stage is succeeded by pines, seedlings of which become established between the clumps of grass. Within 10 to 15 years, the pines shade out the grass and dominate for 70 to 80 years. The pines, however, do not reproduce in their own shade and are gradually replaced by oaks and hickories, which have developed gradually in the lower strata of the forest. After about 150 to 200 years, the oak-hickory climax is again attained, with a few scattered pines remaining as relics of the preceding stage in the succession.

Similar succession commonly occur on western grasslands following overgrazing or drought. Studies on western prairies, after extensive periods of drought, have shown that as the dominant grasses die out they are replaced by early annual grasses and annual and perennial broad-leaved weedy herbs. Unless recovery is hindered by overgrazing, these in turn are slowly supplanted by the climax grasses. Secondary successions are so common that the city dweller need not depart from his immediate environment to study them. They may be observed in vacant lots, along roadsides, and in bare spaces on the front lawn. Vigorous weeds may easily suppress the more desirable lawn grasses.

A knowledge of the principles of succession has been of the greatest value in the management of forests, ranges, agricultural lands, and wildlife areas. As a result of succession, our landscapes are in a process of continual change. Many of these alterations may be observed in one's own lifetime; others are so slow that men living their whole lives in the same region scarcely notice the changes that are taking place. Vegetation as we see it today, then, is not merely a haphazard covering of the land with species of plants. It represents on orderly process of colonization governed by the interaction of plants with their changing environments.

Other biotic factors. The emphasis in the preceding discussion has been placed chiefly upon higher plants as biotic factors. But lower plants are also known to influence plant growth; soil fungi, for example, play an important role. Many of these fungi penetrate the cells of the root, and the threads of the fungus may also be aggregated into a feltlike sheath around the smaller roots. Fungi are almost universally present in soils, and seem essential to the growth of pine, beech, and other trees. Other unique and interesting relationships between plants exist.

Although plants are important as biotic factors, man and other animals are of equal, sometimes of greater, importance as agents which influence plant growth, reproduction, and distribution. Some of the effects of man's activities upon vegetation, such as lumbering, fires, and the grazing of domestic animals, have been indicated. The nucontrolled grazing of cattle, horses, sheep, and goats interferes with forest reproduction and destroys the native cover of grasslands, thus causing soil depletion and erosion. Rodents use seeds as food and thus diminish the reproductive capacity of plants. They also consume leaves and underground stems and injure the underground parts by their burrowing activities. Locally they may be important factors in range depletion.

Among the beneficial effects of animals is their role in the dissemination of seeds and fruits, the part played by insects in pollination, and the contribution of earthworms in increasing aeration, water absorption, and soil fertility. Many other examples of animals as biotic factors could be cited.

The Principal Plant Formations

The geological, climatic, and biotic factors examined thus far in this chapter have. over long periods of time, produced typical vegetation patterns over large sections of the earth's surface. The widespread effects of differences in temperature and moisture are modified by soil and biotic factors to produce four general types of vegetation, all of which are found in North America. These types are described in terms of their climax plants or, in more detail, in terms of the climax associations which develop.

These major types of plant cover may be designated as:

1. tundra,
2. forest,
3. grassland, and
4. desert.

Intermediate between certain of these are other groupings, such as scrubland and savanna—that is, grassland with scattered shrubs or trees. In North America, the precipitation over deserts is roughly 0 to 10 inches a year; over grasslands 10 to 30 inches, and over forested areas 30 to 90 inches. The general limits of the great zones of vegetation found in North America are shown in Figure 5.5. Following is a brief consideration of each of they zones.

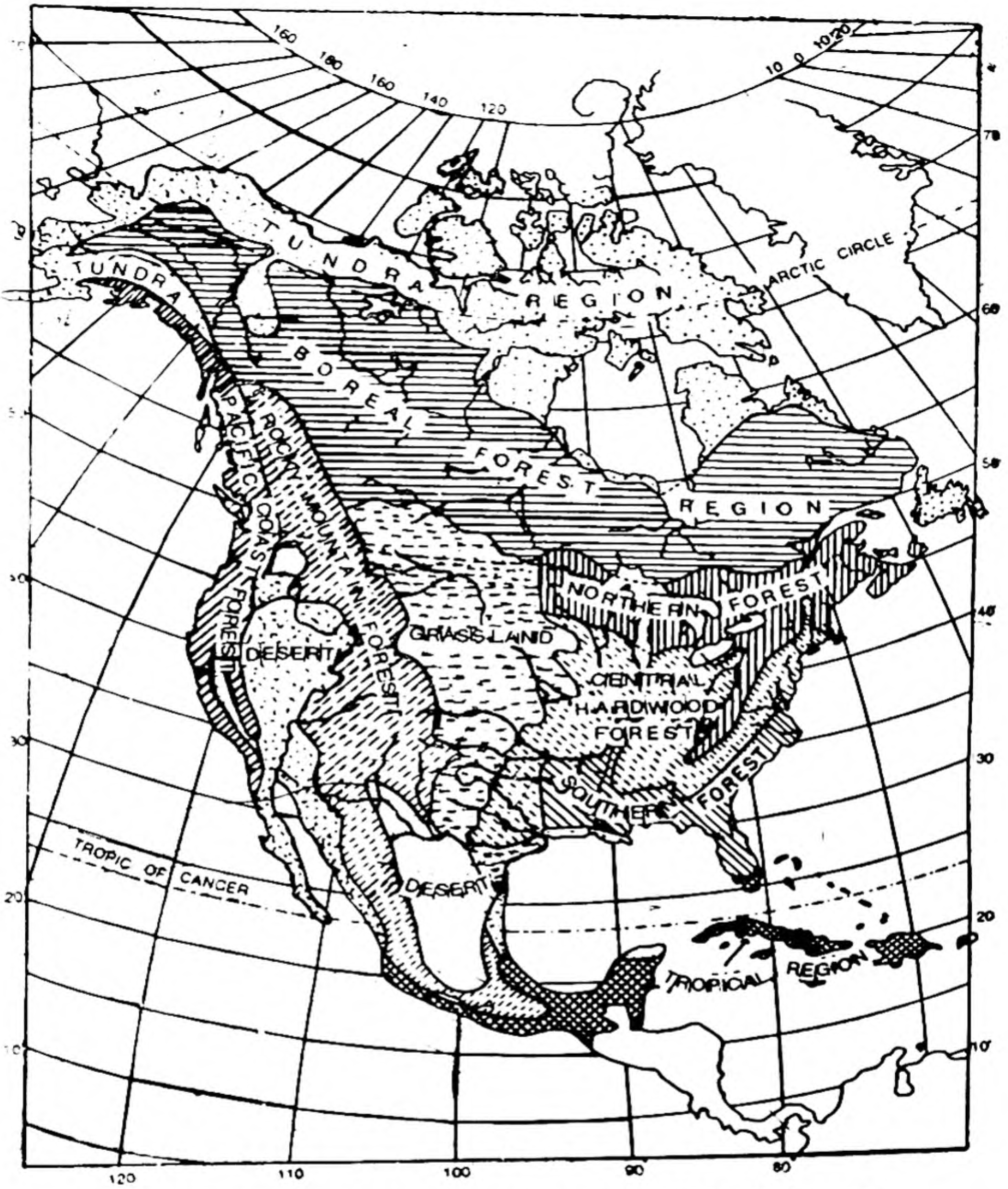
TUNDRA REGION
TUNDRA
BOREAL FOREST REGION
ROCKY MOUNTAIN FOREST
PACIFIC COAST FOREST
DESERT
GRASSLAND
NORTHERN FOREST
CENTRAL HARDWOOD FOREST
SOUTHERN FOREST
DESERT
TROPICAL REGION
ARCTIC CIRCLE
TROPIC OF CANCER
160
180
160
140
120
10
0
10
20
70
60
50
40
30
20
10
120
110
100
90
80

FIG. 5.5: Map of the zones of vegetation of North America.

Tundra

The tundra zone extends from the northern limits of the forests to the limits of perpetual snow. Temperatures of the soil and air are low, and strong, drying winds prevail. The growing season is very short, from a few weeks to two months. The summer days are long, but the light is of low intensity, owing to the inclination of the sun's rays and to fogs, clouds, and drizzling rain. As a consequence of the unfavourable environment, the region is largely treeless. The vegetation consists chiefly of mosses and lichens, grasses, sedges, and dwarf shrubs. Similar conditions may prevail at high altitudes, such as mountaintops above timberline. The appearance of the vegetation in these local areas is very similar to that of the tundra, which extends for hundreds of square miles across the northern parts of this continent, Europe, and Asia.

Forest. The forested areas of North America occur in three great belts—the Boreal, Eastern, and Western forests.

Boreal forest. This most northern forest, which extends all the way across the continent, is primarily coniferous. Spruce and fir are the most important trees, but pine, cedar, and larch also occur. The hardwoods, or broad-leaved trees, are represented by aspen, poplars, and birches. The climate in this region is rigorous, but the growing season is sufficiently long to permit tree growth. The Boreal forest grows largely on thin-soiled, glaciated land, studded with lakes, ponds, and bogs. The plant succession in the ponds and bogs is characteristic. Sphagnum mosses gradually fill the ponds and convert them into peat bogs, where sedges and acid-soil heaths, such as cranberries, blueberries, leatherleaf, and sheep laurel, take over, to be followed by spruce and larch. Such bogs are also found in the northern part of the United States.

Eastern forest. The Eastern forest extends from the east coast across the Mississippi to the Great Plains. It, in turn, has been classified into a Northern forest, Central Hardwood forest, and Southern forest. These are the rickest in the temperate zone in numbers of species of trees (approximately 600). The Northern forest includes coniferous trees, such as red and white pine, hemlock, spruce, and fir, and deciduous trees, such as the birches, beech, maple, basswood, and the oaks. The Central Hardwood forest is largely deciduous. Among the important trees are the oaks, hickories, maples, ashes, black locust, yellow poplar, and walnut, together with other species of great commercial value. The Southern forest is composed of mixed hardwoods and conifers. It includes several species of pine which yield the turpentine and rosin of commerce, red cedar, gums, oaks, hickories, cypress, and others.

Western forest. The Great Basin, arid and hot, divides this forest into two sections—the Pacific Coast forest and the Rocky Mountain forest. Both of these predominately coniferous. Important trees of the Pacific Coast forest are firs and pines, hemlock, cedar redwood, Douglas fir (not a true fir at all), and the spruces. The yellow, or Ponderosa, pine is perhaps the most important species of the Rocky Mountain forest, but Douglas fir and several other valuable conifers occur over wide areas.

To these forest types may be added the Tropical forest, found, in the United States, only in the southern tip of Florida. The species found here, which include mangrove, palms, wild figs, and mahogany, have extended northward from their natural ranges in the West Indies and Central and South Ameriea.

Grasslands. Extending westward from the forested areas to the Rocky Mountains lies a vast, largely treeless area, variable in temperature and generally low in rainfall. Of this region, the part bordering upon the Eastern forest is the prairie, once covered with tall grasses, but now the intensely cultivated western corn belt. This area merges into the Great Plains, once covered with drought-enduring short grasses, such as grama and buffalo grass, now extensively planted to wheat. Grasslands are found also in Washington and California.

Over much of the Great Plains area the 10 to 30 inches of precipitation is concentrated in the early part of the growing season. As a result of the low precipitation and its distribution and the high rate of evaporation, the climate does not favour the growth of trees. Recurrent drought and misuse of the land by overcropping and overgrazing have led to wind erosion over much of the Great Plains area. Large parts of this region are unsuitable for use by man except for the carefully controlled grazing of cattle and sheep.

Desert. Desert regions are markedly low in rainfall, with a high rate of evaporation and low relative humidity. Frequently, high day temperatures alternate with low night temperatures. In North America, the chief desert regions lie between the Rocky Mountains and the Cascades and Sierra Nevadas. They extend southward into the highlands of Mexico.

No sharp boundries exist between deserts and grasslands. The desert usually supports a meager yet interesting vegetation, for the most part clearly adapted to the enviroment. Low evergreen or deciduous small-leaved shrubs are perhaps the predominant life forms. Among other kinds of desert plants are the succulents, such as cacti; the yuccas, with their elongated narrow leaves; wiry grasses; and ephemeral annuals, which germinate quickly, grow rapidly, and mature their seeds during the brief rainy periods.

This chapter has, in large part, dealt with adaptations—variations in structures or processes associated with some particular mode of living. It was at one time considered that adaptations arose in response to the requirements of organisms for survival, and adaptations are frequently so closely correlated with environmental factors that it is difficult to avoid the conclusion that plants have purposefully responded to environmental stimuli. Adaptations are now believed, however, to arise by the selective action of environmental factors upon hereditary variations in the organism. A basis for understanding how these mechanisms operate is presented in the next two chapters.

SOIL

In the normal land-growing (*terrestrial*) plant—and this includs nearly all the important plants from man's point of view—the soil forms the environment of the roots.

In many localities, the soil has been formed from the rocks on which it is immediately situated. In its original state, the land was composed of nothing but bare rock. Of course, some such rocks are seen exposed even to-day. By various means, which will will be examined shortly, most rocks have crumbled or have been *eroded*, thus producing the soil particles. Naturally, various soils are produced according to the various rocks from which they are formed.

As one would expect, there is no hard and fast line between tųe top soil and the rocks beneath from which it has been formed. However. between the reai top soil and the rocks there is usually a more or less definite layer composed of stones intermediate in size between the large boulders of the rock and the very much smaller soil particles. This layer is referred to as the subsoil The three layers, (a) rock, (b) subsoil, (c) soil, can be early distinguished where it is possible to examine the soil in profile, such as on the edge of a quarry or the top of a cliff.

The soil and the subsoil layers vary considerably in different localities. the thickness of soil being dependent upon the hardness of the rocks and the amount of erosion that has taken place. Usually the thicker the layer of soil, the more fertile it is from the point of view of plant production, wild or cultivated The soil is generally rich in humus and in the mineral elements essential for the nutrition of the plant; its texture, too, is more favourable for the growth and functioning of the roots. In these characters it differs from the infertile subsoil.

Erosion of rocks. resulting in the formation of soils, is dependent upon several factors. The main factors are those concerned with the weather; and their effect is often referred to as *weathering*. Rain, sun and frost all play their part. For examaple, the rain gets into the crannies of the rocks; then frost may occur. This causes the water to freeze, which results in an increase in volume. This has the effect of pushing the pieces of rock apart.

Chemical weathering may also take place as a resuld of the solution, oxidation, or hydrolysis of the rock components. Biological weathering also occurs: for example, growing plants and animals help in the making of soil. Charles Darwin showed that earthworms are very effective in bringing up soil particles from the lower layers of the surface, thus turning the soil just as it is turned in digging and plounghing operations.

The formation of all soils depends ultimately on the processes of weathering just described. Many soils have been formed, as described, directly from the underlying rocks. In many cases, however, the soil is not related to the underlying rock and has. in fact, been transported from another locality. An example of this is the boulder clay. formed by the action of glaciers on rocks, and carried as glacial drift for long distances. In regions with such boulder clay, the soil which has been formed on top of this material has no relation to the underlying rocks. Alluvial deposits offer another example of such transported soils.

Weathering processes thus give the mineral skeleton of the soil. Further changes, however, take place. The most important of these is the addition of humus. This is derived from fallen leaves on the surface of the soil and from dead roots in the soil. These are acted upon by a very varied microfauna and microflora, the latter consisting of algae, fungi and, in particular. bacteria. There may be as many as 1,000 millions of these latter in a gramme of soil. They are concerned with the process of nitrification and with the fixation of atmospheric nitrogen. They are also the main agents in converting organic debris into humus. This is a black, colloidal substance derived largely from the cellulose and lignins of the plant remains, and it is of the greatest importance in the formation of soil suitable for plant life. It gives a colloidal-gel coating to the soil particles which holds water, adsorbs calcium and other bases, and aggregates the soil particles into larger particles called *soil crumbs*.

Under natural conditions, the soil thus formed from mineral particles and humus becomes stratified by climatic agencies, mainly by the downward percolation of water the water carrying fine particles and dissolved salts down to lower levels.

This stratification gives what is called a *soil profile*, consisting of a number of characteristic layers overlying the parent material. Soil types may be classified on the basis of their profiles, which can be investigated by digging a trench down to the parent material. In Britain, there are two main types. One is the *brown earth*, formed in regions with moderate rainfall and temperature, such as in most of our deciduous wooldland areas. In this type, the washing out, leaching, from the upper layers is not excessive. The surface layer is rich in bases and in humus and the soil is very fertile. The other type is the *podsol*, characteristic of the cooler and water regions in the north and west of Britain. Under these conditions, the humus-forming bacteria are absent and the plant-remains collect as acid peat on the surface of the soil. Acid wetter, percolating downwards through the soil, leaches the upper layers, which thus become deficient in basic salts and are often grey in colour as a result of the removal of the sesquioxides of iron. Such soils support coniferous forests, moors or healths.

It should be noted, however, that cultivation destroys such stratification, and the soils of fields and gardens are more or less uniform down to the subsoil.

Composition of Soil

The average garden soil which may be considered a good one should contain the following ingredients, the numbers representing percentage by volume:

Rock particles	40
Water	25
Air	25
Humus	10

The rock particles constituting the actual soil vary in size according to the rocks from which they have been formed. The three most common types of soil formations are those in which sand, clay or calcareous material predominates, namely, sand-stones, clays and limestones. The average soil, of corse, is composed of a mixture of these the percentage of each varying with different localities. Then, in many cases, the soil is further characterised by the presence of other substances. For example, the very white, limy (or calcareous) soils of Kent and Sussex are due to the presence of chalk. The red soils of Devonshire and certain parts of Somerset own their colour to the large amount of iron salts in the soil, and the fact that they are formed from red sandstone. Soils very rich in humus are usually dark in colour. Soils containing a great deal of lime are alkaline in reaction, whereas those which contain excess water and humus, such as bogs and marshes, are very acid in reaction. All this is important from the point of view of the plants which will colonise these soils, for certain plants must have very acid soils, whereas others must have alkaline soils, etc.

The largest particles in the soil are the *stones* and *gravels.* With too many such large particles, the soil is very poor, since it cannot retain water. *Sand* forms very coarse soil particles *Clay*, on the other hand, is composed of very fine soil. particles. Intermediate between sands and clays are those particies called *silt*.

A typical soil contains a certain percentage of all these various soil particles, the percentages varying with different localities. A soil which contains chiefly sand and silt, with about 6 per cent of clay, is called a *sand soil*, If it contains more than 25 per cent of clay it is said to be *clayey*. Between the two, we have the more common type of soil, with plenty of sand and clay. Such a soil is called a *loam*. *Sir John Russell* gives the following as being a typical loam: clay, 6-15 per cent; silt, 40-60 per cent; and sand, 20-50 per cent. In an exceptional amount of calcium be present in the soil in the form of calcium carbonate, such as limestone or chalk, the soil is called a *marl*. A marl is usually a rich soil, since the calcium it contains serves a double purpose. First, calcium is one of the raw elements required in the plant's nutrition. Secondly, calcium carbonate is an alkali, and thus prevents the soil from becoming acid or sour.

Clay is colloidal in nature, and therefore, is a very high water-retaining capacity. Drainage from clayey soils, therefore, is very difficult. Naturally, holding the water so tenaciously as a clayey soil does, it is very badly aerated and is heavy. Also, since it is saturated with water, it tends to remain cold. Therefore, a very clayey soil is heavy, badly aerated and water-logged. Crops grown on such soils are usually poor and slow to ripen. In agriculture and horticulture such soils are very often improved by adding larger particles such as sand or coal ash.

Sandy soils are the reverse. They allow an easy percolation of water, and therefore, remain very dry, sometimes too dry. They are also very well aerated, and therefore extremely light. Crops grown on very sandy soils sometimes suffer from the want of water, etc., so a sandy soil is really not much better than a clayey soil, although it is the reverse in composition. Such soils are usually improved by adding heavy farmyard manure, for with such easy water drainage through a sandy soil the leaching of mineral salts goes on at an excessive rate.

It is quite clear that, on the average, loams are the best soils for crops, etc. A rough mechanical way of showing the constituents of loam is to stir a little of such soil in a beaker or glass of water. The heaviest rand particles will soon settle to the bottom of the glass. Closely following the sand will be the silt which will settle above it. The clay particles, on the other hand. being very small and colloidal, will remain suspended in the water, whereas some of the humus will float. The alkalinity or acidity can then be examined by testing the reaction of the water. The colour of the soil is an important physical property for a dark soil absorbs more heat from the sun's rays than a light soil does. Thus, the temperature of a dark soil is higher than that of a light soil under similar conditions. The temperature of the soil is also affected in two other important ways:

(a) degree of slope towards or away from the sun;

(b) amount of evaporation of water from the soil.

Evaporation from a surface has a cooling effect on the surface, therefore the more the evaporation takes place, the cooler does the soil become. Naturally, a very loose, sandy soil has a much higher degree of evaporation than a compact clayey soil.

The rate of passage of water through the soil is also of great inportance. The soil water is usually present on the surface of the soil particle, Passage through the air channels of the soil is due to *capillarity*. It can easily be shown that the smaller the bore in a glass tube, the greater is its capillary attraction. Place several tubes, with very small but varying bores, standing in water. By capillarity, the water will pass up the tubes, but the height to which it rises varies inversely as the diameter of the bores of the tubes. Therefore, the smaller, the diameter, the greater the capillarity. Thus, in soils, passage of water will be greatly affected by the soil practicles. The smaller the particle (for example, clay) the smaller the air spaces, and therefore, the greater the capillarity. The result is that water moves upwards by capillary attraction much more quickly in a clayey soil than in a sandy one.

The physical properties (such as size of particles), chemical properties (such as water-content, mineral-content), and biological properties (such as the soil bacteria) of soils are all of the utmost importance to plants.

VARIOUS TYPES OF PLANTS

Mesophytes

Many habitats provide the conditions usually regarded as those favourable for plant growth, for example, plentiful water, favourable soil factors, and the absence of conditions which might lead to excessive transpiration. The plants growing in such habitats have what we regard as 'normal' structure, and they are termed *mesophytes*.

On the other hand, many habitats present conditions deviating in some way from the favourable ones described above. The plants of such habitats tend to show special adaptations, as in the types which will now be described.

Epiphytes

Some plants, instead of growing on the soil, that is, instead of leading a *terrestrial* mode of life, grow attached to other plants, most frequently growing on the trunks of trees, or in the axils of their branches.

All such plants are termed *epiphytes*. In Great Britain and other temperate countries, epiphytes are represented only by certain algae, such as *Pleurococcus* on the bark of trees, many lichens and some mosses, though certain mesophytes, such as dandelions, sometimes grow epiphytically in the forks of trees. It must be remembered that epiphytes are not parasites, like the mistletoe, for they only use the actual tree, etc., as a support, and not as a source of nutrition.

In tropical countries, especially in the luxuriant vegetation of the jungle, many ferns and flowering plants are epiphytic. Certain orchids are. They cling to the supporting branch of the tree by means of short *clinging roots* which twine round it. They absorb nutrition from the humus (chiefly dead leaves) which collects around them, by means of *absorbing roots*. Many of these plants have special methods of obtaining water. The commonest one is by means of long *aerial roots*. In the Tropical House at the Royal Botanic Gardens at Kew, many of these long aerial roots can be seen suspended from the epiphytic plants growing on the tropical trees there. Other epiphytes have some of their leaves modified as water-collecting pitchers, into which their adventitious roots grow. Many epiphytes have water absorbing hairs and scales, particularly on their leaves.

Xerophytes

Xerophytes are plants growing in habitats where water is scarce or difficult to absorb. The majority of disert plants, such as cacti, are xerophytic. Pine trees also are subjected to physiologically xerophytic conditions, since the water supply is often frozen and then unavailable. The various methods of adaptation, both with regard to the storage of water and the prevention of excess transpiration, have already been examined.

Hydrophytes

Special peculiarities in structure are found in plants which grow in water (called *hydrophytes*). The majority of them, such as the bladderwort and spiky water milfoil (*Myriophyllum spicatum*), are able to absorb water all over their surfaces. The requisite gases, oxygen and carbon dioxide, are absorbed from the surrounding water in solution, just as in the case of seaweeds, etc., which are submerged in the sea.

Many hydrophytes have their leaves floating on the surface of the water, such as in the case of the yellow water-lily (*Nuphar lutea*), white water-lily (*Nymphaea alba*), some species of pond-weed (*Potamogeton* species), frogbit (*Hydrocharis morsus-ranae*), etc. The adaptation of the leaves to this habit, especially with regard to the position of the stomata, has already been considered. Floating leaves carry out gaseous interchanges through their stomata as in land plants and not in solution. All their stomata are on the upper surface of the leaf. But such leaves run the risk of being flooded with water especially when the level of the lake of river rises. Certain adaptations prevent this. For example, the floating leaves of *Potamogeton* are borne on very long stems which thus allow for changes in water level. The petioles of the floating leaves of certain tropical hydrophytes are shaped like a corkscrew, and therefore, in order to remain floating, stretch or contract according to the rise or fall in water-level.

A very exceptional type is seen in the tremendous leaves of the tropical hydrophyte, *Victoria regia*, the giant waterlily of the Amazon. This plant is often cultivated in botanic gardens. There is a special *Victoria regia* house at the Royal Botanic Gardens, Kew, where the plant can be seen. It can only be seen at certain times of the year, since it is an annual. In this, case the floating leaves are so large as to be able to support a baby. Flooding over the top of the leaf is prevented by a vertically growing margin, which is two to three inches high, thus making the leaf look like a floating tray.

Certain hydrophytes grow rooted in shallow water, in which case some leaves grow below the surface of the water, whereas others grow upright above it. The latter are normal in structure. The former, on the other hand, are modified in relation to their submerged position. For example, stomata are absent. Also, they are subjected to currents of water which might easily tear them. How completely submerged leaves adapt themselves to this contingency was seen in the case of the bladderwort. In this case, the leaves become finely divided. In the case of plants with submerged leaves and also leaves above water, the submerged ones *only* become divided. Thus, in such plants, there are two types of foliage leaves in one and the same plant. Common examples of this in Great Britain are the arrowhead (*Sagittaria sagittifolia*), with upright aerial leaves bearing an arrow-shaped lamina and submerged leaves, long and ribbon-shaped; and the water crowfoot (*Ranunculus aquatilis*) (Fig. 5.6), with lobed floating leaves and finely divided submerged leaves. One species of pondweed, the various-leaved pond-weed (*Potamogeton gramineus*), has oblong floating leaves and narrow, linear submerged leaves.

In relation to the difficulty that hydrophytes experience in obtaining air for respiratory purposes, the tissues of the stems, roots and leaves often have very large intercellular spaces, usually in the cortex of the stem and roots. These large intercellular spaces act as air chambers, and this aerated tissue is usually called *aerenchyma*. Such tissue is common in the petioles and roots of the water-lily, and in the stems of *Potamogeton*, and marestail (*Hippuris vulgaris*) (Fig. 5.7).

Plants growing in swaups may be considered as semi-

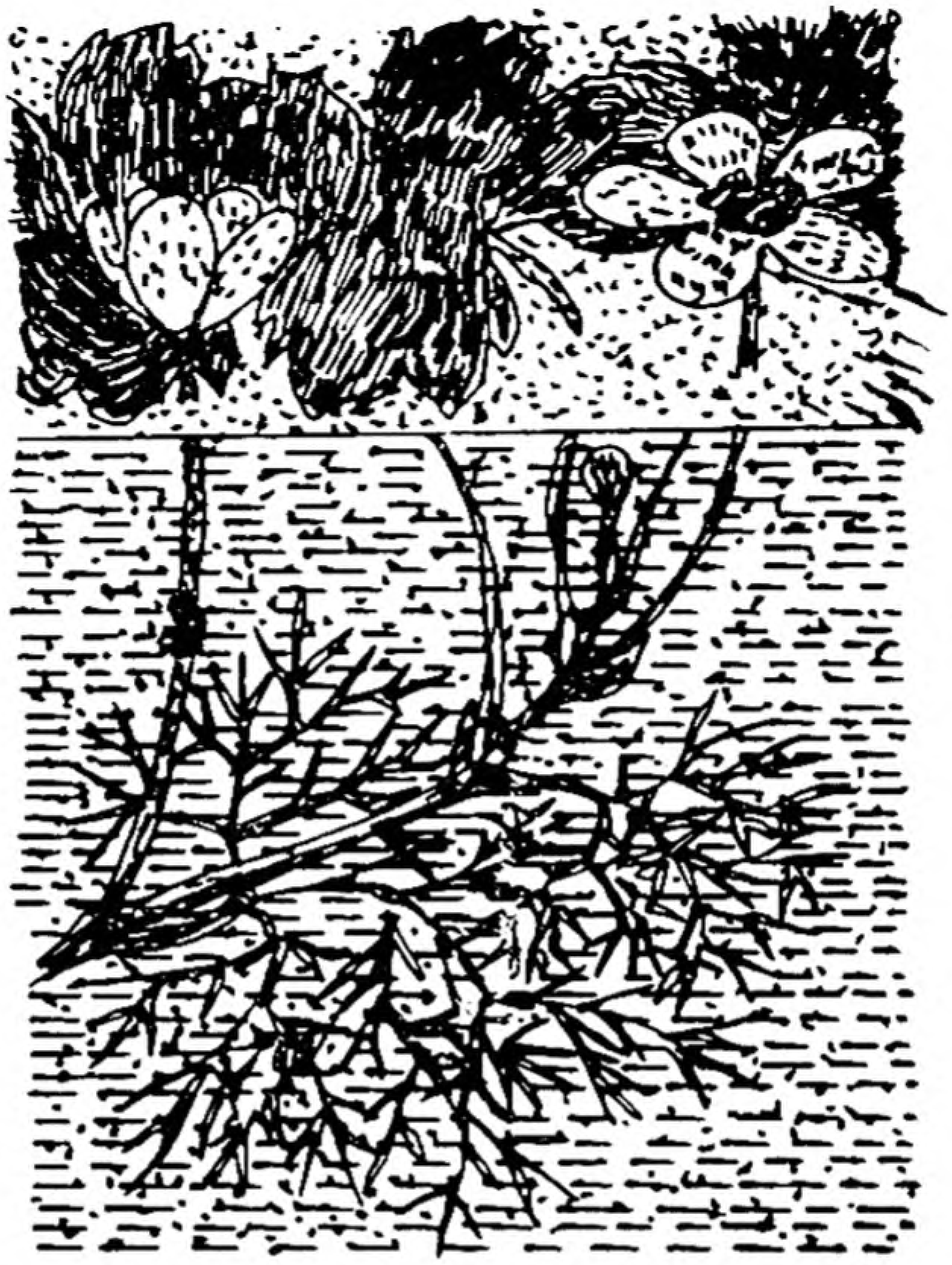

FIG. 5.6: Water Crowfoot (Ranunculus aquatilis). Showing floating leaves, submerged leaves, and flowers.

hydrophytes, since their roots are subjected to water-logged conditions. Many such plants produce respiratory roots, which grow up into the air in order to allow gaseous exchange.

Halophytes

Quite a number of flowering plants are capable of living near the coast where the soil is periodically swamped with sea-water, and several flowering plants can grow in shallow sea-water. Such plants thus adapted to salt water are called *halophytes.* Between high-tide mark and the sand dunes further inland may be found the sea rocket (*Cakile maritima*), salt-wort (*Salsola kali*), etc.; all are halophytes with succulent leaves, and in some cases succulent stems. On tracts of tidal mud, where the soil is constantly saturated with sea-water, may be found the glasswort, which has very minute leaves, but thick, fleshy, jointed stems. The glasswort (*Salicornia stricta*) is often found growing practically alone in salt marshes just out of reach of the neap tides. The succulence of halophytes is not fully understood. Their root-hairs have an extremely high osmotic pressure, so that the plants can absorb water even from a soil saturated with sea-water. The significance of the storage of water in the succulent tissues of the aerial parts is, however, not understood. The branched stems of the glasswort can absorb dew and rain-water readily. The eel-grass or grass wrack (*Zostera marina* and *Zostera nana*) is a land plant which has become totally adapted to marine aquatic conditions. The entire plant, including flowers, is submerged. It spreads by means of a creeping stem, and bears ribbon-shaped leaves sheathed at their bases. *Zostera marina* suddenly mysteriously disappeared from the coasts of Britain; but there are localised signs of its return.

Of great value to man are certain halophytic grasses. These are capable of growing in salt marshes, and they grow so prolifically that finally the marshes become dried up, and their levels raised, owing to the great activity of the plants in forming humus from their own dead bodies. In Europe, one halophytic grass, *Spartina maritima*, occurs in the salt flats of southern England, and in the Mediterranean coasts. In the salt marshes around Southampton is another species, *Spartina alterniflora*, where it was introduced by ships from America. There it is colonising the marshes so much that certain parts around Southampton, which were once marshes, are now dry land.

The most efficient halophyte in colonising salt marshes is the rice grass, *Spartina townsendii*, a hybrid of *maritima* and *alteoiflora*. This halophyte originally grew around Southampton, but now it has spread along the mud flats of the southern coast, and is choking up certain parts of Poole harbour, in Dorset. Rice grass is being used for the reclamation of new land from the sea. This is the case in Holland, where, since 1924, wher

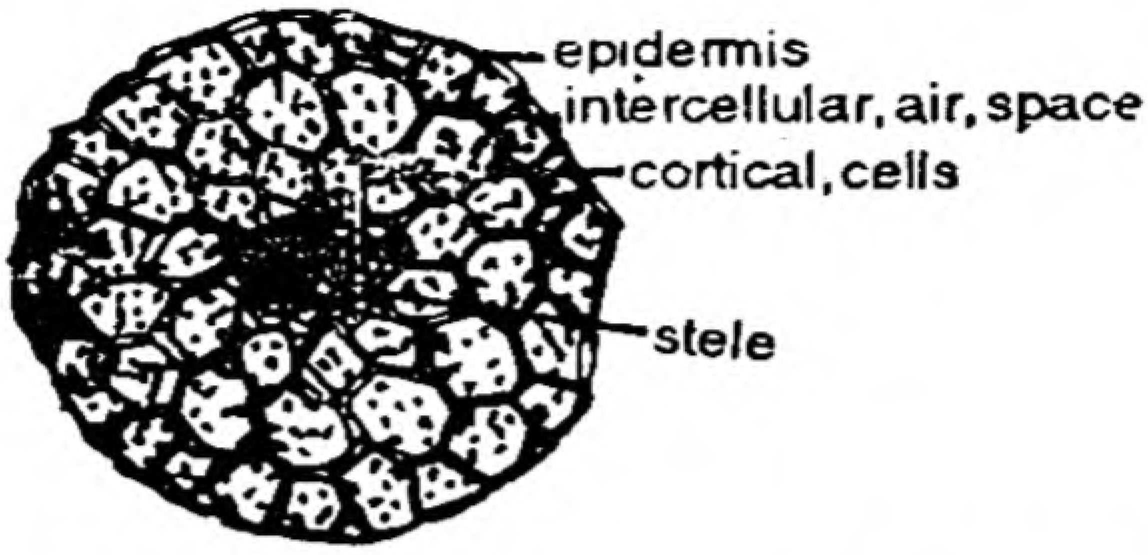

FIG. 5.7: Photomicrograph of Transverse Section of Marestail. (T.D.T. Hall.)

the plant was definitely introduced for the purpose, it has grown so prolifically that acres of land, previously salt marshes or actually under the sea, have been reclaimed and now used for agricultural purposes.

Competition and Colonisation

The study of the social life of plants can be made a very extensive one, taking in the examination of the distribution and inter-relationship of plants throughout the world. This type of study, dealing mainly with plant distribution, is described as *plant geography*. Ecology, however, is concerned with the relation of plants, either as individuals or as communities, to their habitat. Communities of plants behave in many ways like individuals, and thus have an origin and a developmental history.

One very important thing to realise is that the seeds of plants in their wild or native state are distributed largely by chance. On any one piece of ground, thousands of plant seeds must fall in a year, yet it is clear that only a few actually take to the soil and grow. If it is a barren, infertile soil, with a low mineral content, bad exposure and unsuitable climate, few plants will succeed. The rest of the seeds perish.

One excellent method of studying colonisation by plants is to watch the gradual invasion of a newly exposed piece of soil. Of course, in Great Britain, this is not very easy; yet one can gather much information concerning plant colonisation by studying the newly made banks of roads and railway cuttings. The study may extend over several seasons, and the type of plant, the time that it arrives, the percentage of different plants, etc.. duly noted.

In such cases, plants which develop quickly from spores, and not seeds, are the first arrivals. Spores are produced by many of the simpler types of plants, which as algae, fungi, mosses, liverworts and ferns. These usually arrive first, since spores are very small and are easily carried in the air. Next come the ephemeral and annual flowering plants common to the neighbourhood. A *community* of plants thus established is still not very closely packed and it is still open to new-comers. It is therefore called an *open* community.

Hardy perennials such as certain grasses, thistles, plantains, dandelions, etc., then become established, and competition is set up between the plants, because, by now, the community is becoming overcrowded. Then the weaker plants are chocked out and die, and the hardier ones become more firmly established. Finally, only those plants which are suited to that soil and climate remain, and then, when the ground is fully occupied, the community is said to be *closed.* This does not necessarily complete the development of the vegetation, for further changes may occur. Shrubs may invade the community of herbaceous perennials and, even tually, trees may become established. Thus, the habitat shows a *succession* of plant communities. If no other factors intervene, the succession will end with the development of the highest type of plant community which the climate will permit. In this country, woodland is what is termed the 'climatic climax', and much of our vegetation would revert to woodland were it not for the action of man and his domestic animals.

At all stages in the development of a plant community there is competition between the component plants for light and air. But there is also competition among their roots for a place in the soil. It is amazing sometimes how far the roots of trees will penetrate the soil; some roots go down more feet than the topmost shoots are high. The root competition is chiefly due to the search for an adequate water supply. It has been shown experimentally that apple trees planted 40 feet apart yielded 43 bushels more fruit to the acre than similar trees planted 30 feet apart. This in interesting from the point of view of plant accommodation in both air and soil.

The largest and most comprehensive type of plant community is the plant *formation*. Such formations are, in fact, the main world types of vegetation. Some are determined by climate, as for example, tropical rain-forests, temperate grasslands (like prairies and steppes), and deciduous forests (such as oak and beech woods). Others are determined by soil factors, for example, reed swamps and salt marshes. Although the general type of vegetation in each formation is the same all over the world, the species composing the formation vary in different regions.

So far as British vegetation is concerned, sown crops and plantations occupy a great part of the country. The remaining vegetation is largely semi-natural, that is, it consists of native plants, but has been more or less interfered with by man's activity. It is practically only the vegetation of the high mountains and some parts of the sea-coast which is truly natural.

The actual units of the semi-natural and natural vegetation are called *associations*. Each is composed of a group of characteristic species, one or several of which have a great effect on the community as a whole and are therefore called the *dominants*. For example, the oak is the dominant plant in an oak-wood; the heather may be dominant on a moor. Smaller component units of an association are termed *societies*. For example, in an oak wood, there may be a society of primroses, or one of bluebells.

Plant Communities

The examination of a plant community makes interesting study.

There are several methods of study. One is to examine a rather large area. Then obtain a blank map and shade in with various colours the different types of vegetation, such as woodland, scrub, meadow, hedgerows, moor, marsh, water, cultivated land, etc. Then this should be correlated with the physiography, the geological formations, the weather and types of animals, etc., all of which have definite effects on the community.

A more intensive study is that referred to as the *quadrat chart* method. This can only be applied to a much smaller area. In this case a rectangular area is marked out on the land by tapes. This is then divided into smaller squares by tapes or sting, then the distribution of the individual plants inside the quadrat is represented on squared paper.

A second method applicable especially to a hedgerow, edge of a wood, river bank, etc., is that called the *transect method.* This involves drawing a section through the habitat to be examined, then representing the various types of plants along the section.

It must be remembered that no plant community is absolutely stable. There are certain factors which decide what type of plant shall constitute it in the first place, such as nature of the soil, climate, etc.; but then there are other factors such as disease epidemics, useful and harmful animals which come along afterwards and modify it. All the factors which influence the nature and development of the community are known collectively as the *habitat factors*.

Such factors are so numerous that it would be difficult to name them all. Also, whereas one certain factor may be very potent in one plant formation, it may be entirely absent in another. However, certain factors are rather general. *Climatic* factors, for example, are very important. These comprise chiefly conditions of rainfall, temperature, light, and prevailing winds. In Great Britain, rainfall is heaviest in the west, and there wet moorlands and marshes are found; in the east of England, where the rainfall is much less, dry heath are common. In the north.

especially Scotland, it is much colder, and this greatly affects vegetation.

Physiographic factors depend upon the shape of the country, mountainous or flat, etc. The slope of the land, too, helps to determine the degree of warmth and light and amount of exposure to the prevailing winds that the vegetation will receive.

The chemical and physical nature of the soil is a very important factor. Such factors associated with the soil are called *edaphic* factors.

Finally, a very potent factor in nearly all cases is the *biotic* factor. This involves the influence of other forms of life, plant and animal. For example, a beech tree casts a very dense shade. The result is that in beech woods very little ground vegetation may be found. Therefore the beech trees are a biotic factor. Many animals form a biotic factor. Some give manure and therefore form a useful biotic factor; other, such as herbivorous animals, for example, rabbits, slugs and snails, eat the plants of the community and are thus a harmful one. Animals such as those used in the dispersal of seeds and fruits, and insects used in pollination, clearly are important factors in plant distribution. Man himself is a biotic factor even where wild plants are concerned. This is well seen in the forests where lumbering goes on.

An intensive study will involve listing all the plants in the community, with notes on the frequency with which each occurs. Since communities show seasonal variations, several studies should be made at different times of the year. First of all one should begin with a note of the type of country. Dates when examined should be stated, and a detailed study of the factors, especially edaphic and climatic, should be made. At all times the ratio and distribution of plants within the association should be thoroughly investigated.

For example, one, two or perhaps even three plants form the most important members of a plant association. These, as mentioned earlier, are called the *dominant* plants. The dominants very often help to answer the question of why are the others such as they are' for dominants are often biotic factors to the other members of the association. This may be seen in a beech-wood association. Here, few herbs are found growing on the soil. Very often, closely associated with the dominant plants are others which are almost as common. They are then said to be *sub-dominant*. Less common again, but evenly distributed throughout the association, are other plants. These are said to be *distributed*. Much less common again are those plants said to be *occasional*, and the sparsest of all are classified as *rare*.

Woodlands

Very familiar plant associations in Great Britain are the *woods*. There are several types of wood associations in this country. In the highest altitudes are wood associations with pine (*Pinus* species) or birch (*Betula* species) as the dominant plants. At lower levels are two types, depending chiefly on the type o soil. On the lower hills with a sandy soil are the dry oak woods, whereas on a clayey soil are the damp oak (*Quercus* species) woods, containing many hazel trees (*Corylus avellana*) as sub-dominants. On the chalk soils at the same levels are beech (*Fagus sylvatica*) woods and, on limestones, the ash (*Fraxinus excelsior*) woods. On the plains, that is the lowest levels of all, the woods are chiefly a mixture of oak and ash, whereas on the very damp soils at such levels the woods are smaller, being composed chiefly of alder (*Alnus gluttnosa*) and willow (*Salix* species).

In all the cases of wood associations mentioned, the trees form the dominant plants. They act as biotic factors in different ways so that each type of association has its special sub-dominant, distributed, occasional and rare plants. For example, the beech wood is very dense, but usually well manured with the human formed of fallen leaves. Abundant on its edges is the bluebell (*Endymion nonscriptus*, May—June*). Distributed

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* Times of flowering are indicated in brackets.

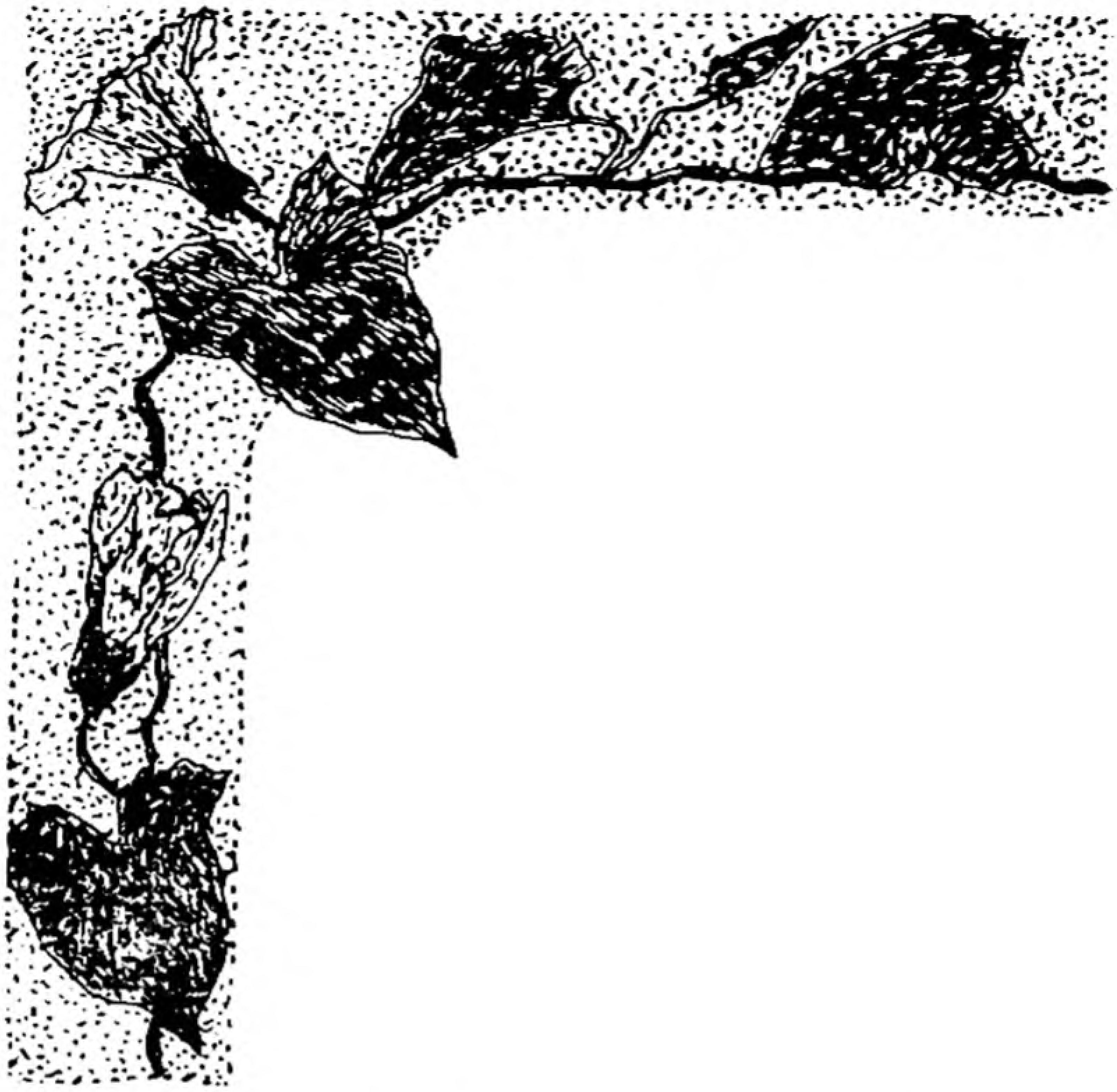


FiG. 5.8: Great Bindeed (Calystegia sepium).

throughout is the bird's-nest orchis (*Neottia nidusavis*, June-July,) a saprophytic flowering plant which clearly will not suffer from the dense shade; but green herbs are rare. In the dry oak wood, high up on the hills, subdominants are gorse (*Ulex europaeus*, all the year round, but mainly February-May and August-September), ling or heather (*Calluna vulgaris*, June-Octaber) and bracken (*Pteridium aquilimum*). In the dry oak wood of lower levels, the subdominants are holly (*Ilex aquifolium*, May-August), wood anemone (*Anemone nemorosa*, March-May), with the foxglove well distributed. The damp oak wood is much more shaded and the herbaceous undergrowth is represented best by wood sorrel (*Oxalis acetosella*, May-June), violet (*Viola canina*, April-August), woodruff (*Asperula odorata*, May-June), etc.

Hedgerows

The *hedgerow* forms a very interesting plant community, since there are several levels to examine, comprising chiefly the hedge itself and the bank. The hedge itself varies in different parts, being composed of some, or all, of the following; hawthorn, hazel, maple, bramble, blackthorn, willow, holly, etc. The bank of the hedge shows more variations. At the top are plants which have to contend with a certain amount of shade. These may be climbers which can scramble to the light, such as the bramble (*Rubus fruticosus*. July-October), clematis (*Clematis vitalba*, July-September), great bindweed (*Calystegia sepium*, June-August) (Fig. 5.8), honeysuckle. (*Lonicera periclymenum*, June-September), black bryony (*Tamus communis*, May-June), white bryony (*Bryonia dioica*, May-September) and various vetches. They have different means of climbing, which are worthy of study. Other plants which can endure the shade of the top of the bank are those with very long, erect stems, such as stinging nettle (*Urtica dioica*, June-September), white deadnettle (*Lamium album*, May-September) but frequently much earlier and much later), certain grasses, hedge mustard (*Sisymbrium officinale*, June-July), garlic mustard (*Alliaria petiolata*, May-June,) greater stitchwort (*Stellaria holostea*, June-August), etc. Finally, there may be plants which prefer the shade, such as certain ferns, broad-leaved garlic or ramsons (*Allium ursinum*, May-July), wild arum (*Arum maculatum*, August October), enchanter's nightshade (*Circaea lutetiana*, June-August), foxglove (*Digitalis purpurea*, June-September), primrose (*Primula vulgaris*, February-May), ground-ivy (*Glechoma hederacea*, March-June) germander speedwell (*Veronica chamaedrys*, May-June), violets, and a host of others.

On the slope of the bank the light is abundant. Here, therefore, we find prostrate plants such as creeping buttercup (*Ranunculus repens*, May-September) wild, strawberry (*Fragaria vesca*, May-July), etc., and rosette plants such as daisy (*Bellis perennis*, March onwards), dandelion (*Taraxaum officinale*, March-September, sometimes earlier, sometimes later), great plantain (*Plantago major*, June-August), etc.

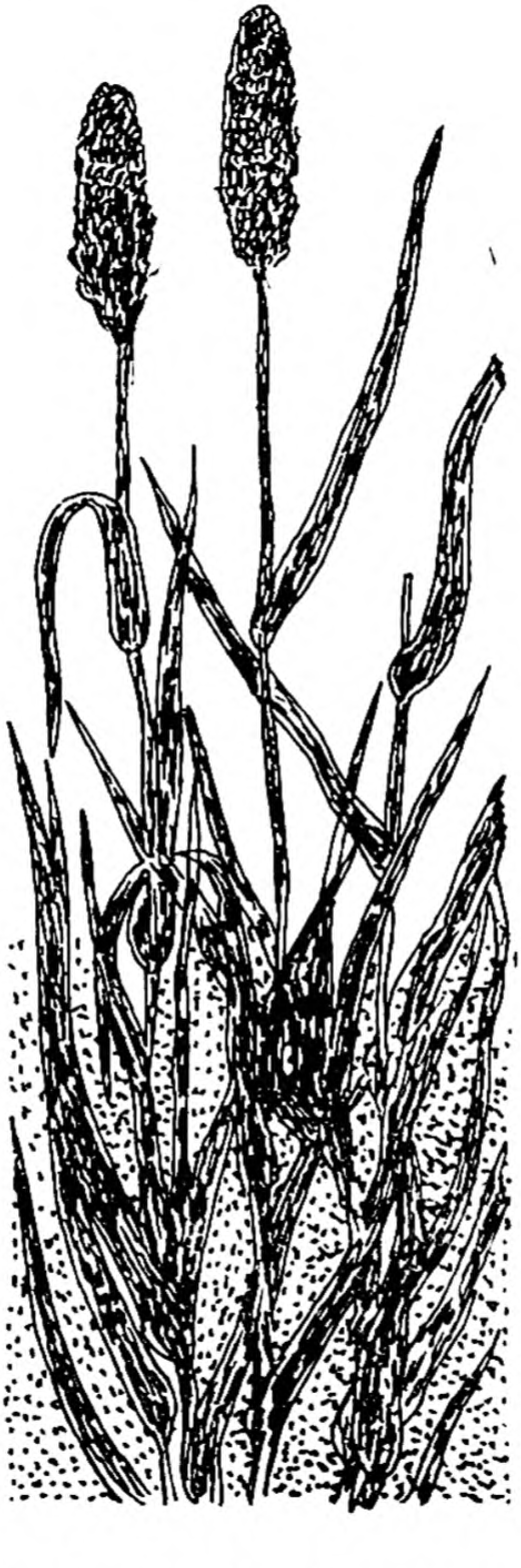


FIG. 5.9: Meadow Foxtail (Alopecurus pralensis).

MEADOWS

In the majority of *meadow* associations the dominants are usually grasses, such as perennial rye grass (*Lolium perenne*. June-July), meadow foxtail (*Alopecurus pratensis*, April-June, Fig. 5.9) and meadow fescue (*Festuca pratensis*, June-August), together with white or Dutch clover (*Trifolium repens*, May-September) and red clover (*Trifolium pratense*, May-September). In various meadows, certain subdominants may be found according to the soil structure and composition. These may be daisy, buttercup, cowslip (*Primula veris*, May-June), common sorrel (*Rumex acetosa*, May-July), ox-eye or moon daisy *Chrysanthemum leucanthemum*, June-August), etc.

Lawns and *arable land* are associations closely related to meadows, with the exception that in these the dominant plant or plants are cultivated. Usually, all other plants, subdominant, distributed, occasional or rare, in such associations, are *weeds*. A weed is any kind of plant which grows where it is not wanted or is a nuisance. Certain meadows have their weeds, since they are required for the production of hay. Any plant growing in such a meadow which will spoil the hay is therefore a weed. Such weeds are chiefly the ox-eye daisy, common daisy, common sorrel, common buttercup (*Ranunculus acris*, June and August). spear plume thistle (*Cirsinm vulgare*, July-August), etc.

On cultivated lawns the dominants are various grasses. The most troublesome weeds of such associations are the rosette plants such as dandelion, daisy and plantain. The yarrow (*Achillea millefolium*, July-August) and creeping buttercup (*Ranunculus repens*, May-June) also form troublesome occasionals. In gardens, the chief weeds are groundsel (*Senecio vulgaris*, February-December), small or lesser bindweed (*Convolvulus arvensis*, June-August), small chickweed (*Stellaria media*, February-October), various thistles, and dandelion. In cornfields they are the same at the early stages of cultivation. Later, along come the charlock on wild mustard (*Sinapis arvensis*, June-August), various buttercups, and, later still, common red poppy

(*Papaver rhoeas*, June-July) and corncockle (*Agrostemma githago*, July-September).

Aquatic and Semi-Aquatic Associations

Marshes, *ponds* and *streams* have very characteristic plant associations. The diagnostic features of some of these plants have already been examined with relation to their hydrophytic habit.

In a marsh the soil only is water-logged, therefore only the roots and rhizomes of the plants are affected by the excess water. In some marshes the dominant is the osier willow (*Salix viminialis*, April-June), whereas in others it is the common rush (*Juncus conglomeratus*, July-August); more rarely it is the great reed mace (*Typha latifolia*, July-August); or common reed (*Phragmites communis*, July-August). Subdominant and distributed include branched bur-reed (*Sparganium ramosum*, June-July), unbranched bur-reed (*Sparganium simplex*, June-July), meadowsweet (*Filipendula ulmaria*, June-September), ragged robin (*Lychnis flos-cuculi*, April-July), wild iris or flag (*Iris pseudacorus*, June-August), marsh marigold (*Caltha plaustris*, April-June), marsh mallow (*Althaea officicalis*, August-September), water mint (*Mentha aquatica*, August-October), water forget-me-not (*Myosotis palustris*, June-August), etc.

Between the marsh and the pond or stream proper comes an intermediate formation, commonly called the *marginal* association. This formation can often be divided into three zones or associations, which merge into each other, provided the slope of the land towards the open water is a gentle one. The first zone is that nearest dry land. Here, only the roots are subjected to water-logged conditions. The members of this zone are similar to those of the marsh, together with others that are dominant or subdominant, such as reeds (*Phragmites* species, July-August), bulrushes (*Schoenoplectus lacustris*, July-August), common rushes sedges (*Carex acutiformis*, May-July), horsetails

(*Equisetum* species), watercress (*Lepidium sativum*), etc. This shore association is often called a *reed-swamp* association.

The next association is that intermediate between this reed-swamp association and the complete water association. In it, plants which are rooted in the soil, with parts of their stems submerged but leaves and flowers held above the water or floating on it, may be found. Such plants are the arrowhead (July-August), water crowfoot (May-August), water-lilies (July-August), *Potamogeton* species etc.

In the third association, the plants are entirely submerged. The most common types found here are Canadian pondweed (*Elodea canadensis*), and tape grass (*Vallineria spiralis*). *Elodea* is of particular interest owing to its prolific growth. It can reproduce itself by a very simple means of vegetative reproduction. Any small branch, if broken off, will reproduce a new plant. So prolific is this, that once *Elodea* establishers itself in a suitable pond, it soon chokes up the pond, unless it is periodically cleared out. That is why this plant is such a nuisance in water-works, etc. The great rate at which it can vegetatively reproduce itself can be realised when one learns that before 1841 it was quite unknown in England. Now it is one of the most common and prolific water plants throuohout the country, and also Scotland and Wales. Actually it is a native of North America and Canada. Then it was mysteriously introduced into Co. Down in Ireland in 1836 and in England in 1841. To-day it is common in nearly every lake, pond, stream and ditch of the islands. Vegetative reproduction is the only means of multiplication of *Elodea* known in Britain. The flowers are unisexual, and in this county the male plant is very rare; so fertilisation is unlikely.

Heaths and Moorlands

The *heath* association may be found on dry, sandy soils with a thin covering of acid peat. The dominant is ling or heather (*Caluna vullgaris*, July-October). In certain areas other dominants are cross-leaved heath (*Erica tetralix*, July-October) and bilberry or whortleberry (*Vaccinium myrtillus*, May-June). Most heath plants are much exposed and therefore have certain xerophytic adaptations. The heather, for example, has rolled leaves. A local subdominant is the grose which, as we have already seen, is xerophytic. Other local dominants are the mat grass (*Nardus stricta*, June-August) and bracken. Occasionals are represented by the Scots pine.

Real *moorland* association differ from the heath in that they have a great deal of a peat in the soil, which is usually very acid. Peat is formed chiefly by the accumulation through hundreds of years of *Sphagmum*, the peat moss, common in the north and west of Britain and also in Ireland. The dried peat forms a kind of fuel. The formation of this deep, acid peat is due to the prevailing low temperatures, lack of calcium, and lack of soil aeration (due to high rainfall). Under these conditions the aerobic, humus-forming bacteria are largely absent. Higher up, wet moors and associations often have cotton grass (*Eriophrum anguttifolium*, June-August) as the dominant. This cotton grass association can be found in the higher moors of York-shire and on Exmoor (Somerset and Devon) and Dartmoor (Devon). Cotton grass is not a member of the grass family (Gramineae), but of the sedge family (Cyperaceae).

The very low-lying moors contain not only peat, but also much water and are very acid. Here may be found certain carnivorous plants such as the sundew (July-August), butterwort (May-July), and, in the free water, bladderwort, (June-July). These make up for the lack of available nitrogen in such soils by developing mechanisms for catching small animals which have a high nitrogen content. Large rushes also abound on such soils.

The wet peat soils of the east of England, especially in Cambridgeshire and Norfolk, vary from this in that they are very rich in mineral salts, especially of calcium, and are therefore alkaline. They are commonly called *fens*, and the plant associations of the Fen District vary accordingly. The formation of peat is here due entirely to the lack of soil aeration. The dominant tree is the alder. The Fen District of East Anglia, right up to and including the Wash, has been the subject of intensive drainage schemes ever since the time of the Roman invasion. Consequently, soil conditions, especially from the point of view of water content, are constantly undergoing changing conditions. Thus has man become a very potent biotic factor in this area. Its detailed ecological study has therefore offered great opportunities for botanists and zoologists.

With changes in conditions, great changes in the flora are taking place. New plants come, old-established ones disappear, dominants become rare, and rare, become dominant. Thus the ecological aspect of the country is constantly changing. The study of this is known as dynamic ecology, as opposed to static ecology. So important is such an area to the plant and animal ecologists that one part of it, Wicken Fen in Cambridgeshire, has been purchased for the nation by the National Trust. Thus has it been secured, for all time, for further studies in ecology, etc.

SAND-DUNES

An important plant formation is that of the *sand-dune*, so common around our seashores. Here, conditions are very exceptional. The almost pure sandy soil, consisting of sand blown up from the shore, is very loose and consequently the surface bayers are abnormally dry, although water (formed by condensation) occurs at a lower level. The surface is exposed to bright light, heat and strong wind. These are factors tending to increase the rate of transpiration.

Sand-dunes usually occur as a series of ridges running parallel to the shore line. Moving inland, one comes first to low hummocks of sand colonised by the sea couch grass (*Agropyron junceiforme*) or by the sea lyme grass (*Elymus arenarius*).

Behind these are the mobile or shifting dunes on which the dominant plant is the marram grass (*Ammophila arenaria*. The sea holly (*Eryngium maritimum*) is also characteristic.

Further inland still are the fixed dunes. These are covered with a more or less complete carper of grasses, and there may be patches of shrubs like the sea buckthorn (*Hippophae rhamnoides*) and the elder (*Sambucus nigra*). Characteristic leguminous plants such as rest harrow (*Ononis reclinata*) and bird's-foot trefoil (*Lotus corniculatus*) are usually present.

Between the lines of mobile and fixed dunes there are dune valleys where the vegetation is often dominated by the creeping willow (*Salix repens*). The flora is frequently very rich, including the wintergreen (*Pyrola rotundifolia*), grass of Parnassus (*Parnassia palustris*), etc.

The series of dunes is interesting, since it represents a definite succesion of vegetation. The mature, fixed dunes have, in fact, all passed through the stages of the low couch grass hummock and the mobile *Ammophila* dune. The building up of the dunes is brought about by the sand-binding capacity of the tangled rhizomes and roots of the dominant plants.

Coastal Areas

Coastal areas offer splendid opportunities for intensive ecological studies, such as that of the sandy seashore, or of cliffs and rocks, salt marshes, etc. Space will not allow full descriptions here. Many plants to be seen in such areas are not confined solely to the coast; but there are some plants which almost are, though even some of these do not show obvious halophyatic adaptation. A brief account of those to be looked for, given in order of flowering throughout the year, follows.

On muddy seashores, the thrift or sea pink *Ameria maritma*) is one of the first plants to bloom, presenting its rose-coloured flowers in April, and so on until September. This symbolic plant is sometimes also known as sea gilliflower; but it is not confined to the seashore, being also an inhabitant of mountainous areas,

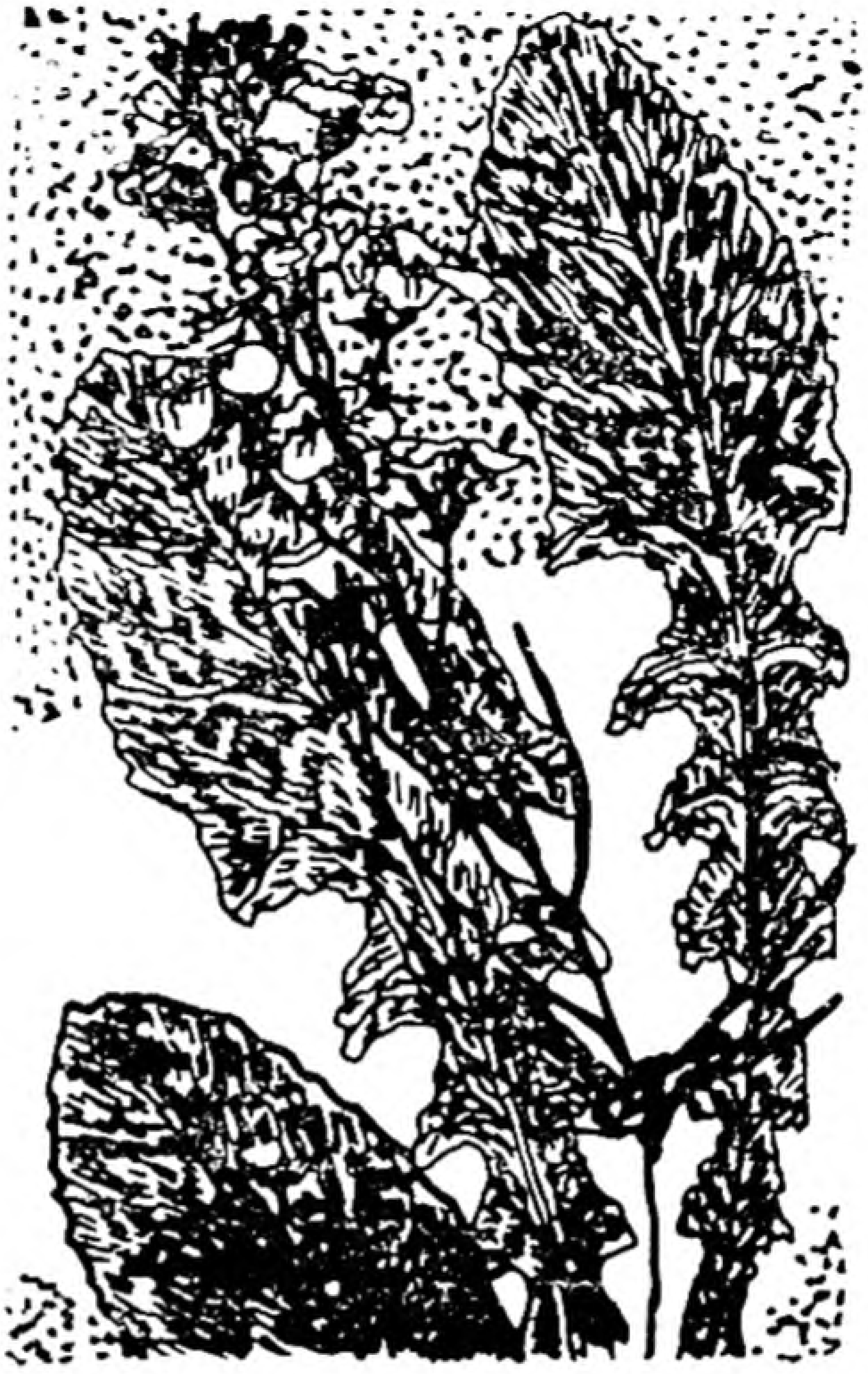


FIG. 5.10: Sea Cabbage (Brassica olcracea).

especially in Scotland. It is compact and has a strong rootstock and grass-like leaves. It is therefore prized by gardeners as a rock and border plant.

A very small member of the cabbage family (Cruciferae), namely, scurvy gross (*Cohlearia officinalis*) is displaying small white flowers during May, and continues to do so until August. It grows four or ten inches high, and betrays its halophytic habit by the fleshiness of the vegetative organs.

The sea pearlwort (*Sagina maritima*) is a tiny member of the pink family (Caryophyllaceae. It blooms during May to September. The flowers are white and the leaves fleshy.

Another member of the pink family, the sea sandwort (*Honkenya peploides*) grows in tangled masses on the seashore, a prostrate plant showing its white flowers during May to September. Its leaves also fleshy.

Of special interest is the samphire (*Crithmum maritimum*), a member of the family Umbelliferae. It flourishes on coastal rocks and cliffs. This plant is much prized by local inhabitants, who like to gather is just before it blooms, that is, late May and early June, sprinkle the fleshy leaves with salt and pickle them. They make good eating—a fact known by seashoremen for centuries. This tufted plant has a strong rootstock, which grows well into the crevices of the rocks. The umbels of yellowish-green flowers are displayed mainly during June to August. The stems and leaves are thick and very fleshy, and the latter are glaucous, that is, covered with a 'bloom' like a plum of black grape.

The progenitor of all cultivated members of the cabbage tribe is the sea or wild cabbage (*Brassica oleracea*), which grows on cliffs and sometimes still further inland, though it is found mainly in the south and west of England. Its large, lemon-coloured flowers appear during June to August. Though the roots are tomewhat fleshy, the leaves are not; but they are glaucous. The lower leaves are lobed and have wavy margins: the upper leaves are sessile and not lobed (Fig. 5.10).

Another rarity, and again a progenitor of a garden plant, is the wild asparagus (*Asparagus officinalis*), which grows on sea cliffs and sometimes inland on waste areas. It belongs to the lify family (Liliaceae). It attains a height of eight to eighteen inches, differing from the cultivated form only in size. Wild asparagus grows abundantly in some parts of Europe; in fact, on the steppes of the U.S.S.R. it is so prolific that cattle eat the young shoots as fodder. The young thick and fleshy shoots bear green or purplish scale-leaves. During June and July taller and more graceful shoots emerge from the axils of the lower scale-leaves. These bear needle-like phylloclades and yellowishgreen pendulous flowers.

On the dunes, the marram grass blooms during June to August. So also does the common bound's tongue (*Cynoglossum officinale*).

In salt marshes, the parsley dropwort *Oenanthe lachenalii*), of the family Umbelliferae, blooms during June to August. Its roots are fleshy, the leaves finely divided and the small white flowers borne in loose umbels.

Among the sea shingle it should not be difficult to discover the sea catchfly or sea campion (*Silene maritima*) blooming with fairly large white flowers during June to August. It is closely related to the bladder campion of the pink family (Caryophyllaceae).

Seaside bindweed or seaside convolvulus (*Calystegia sodlanella*) favours hard sand. It is a near relative of the great

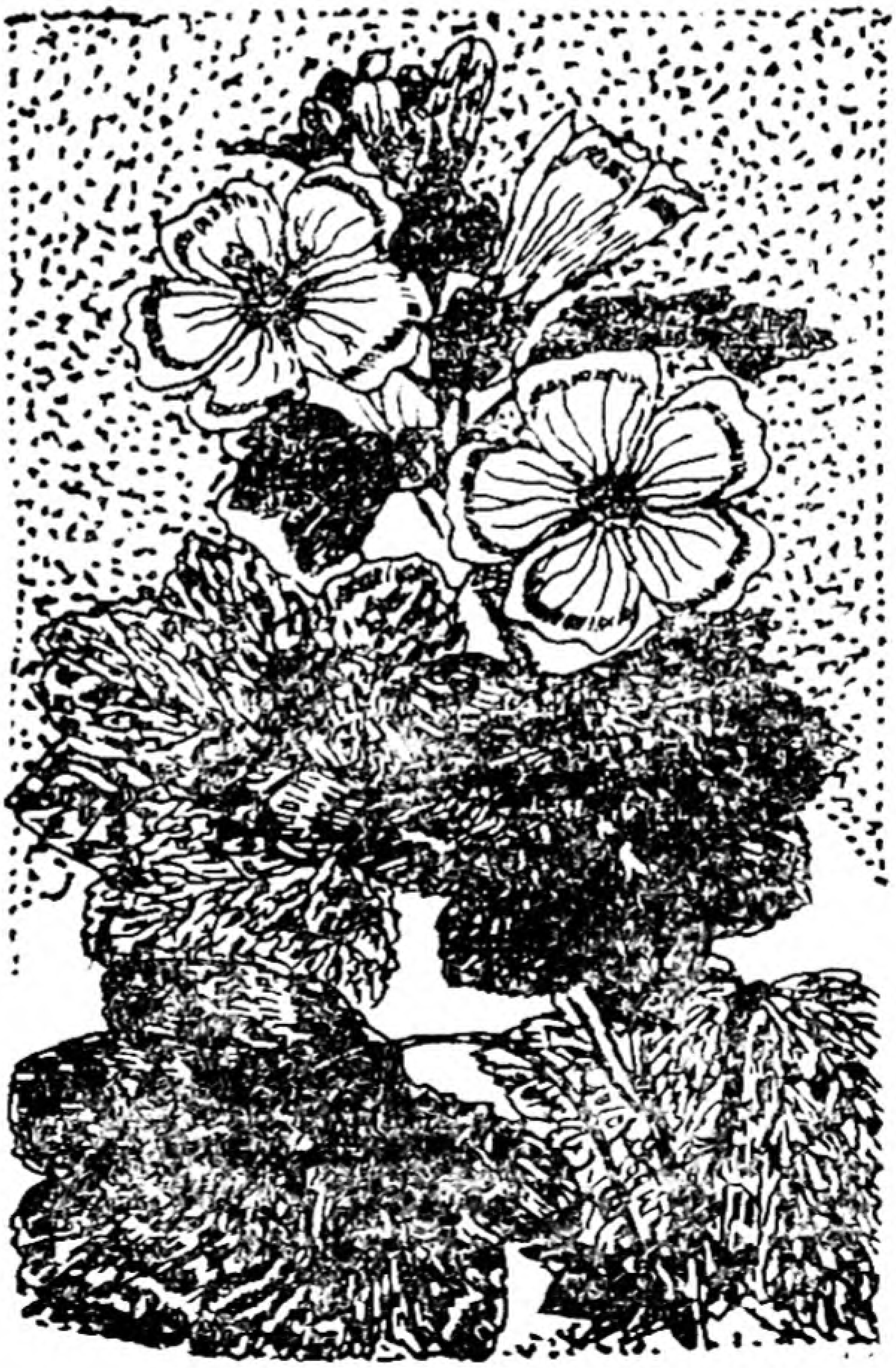


FIG. 5.11: Tree-Mallow (Lavatera arborea).

bindweed. It leaves are small and heart-shaped, borne on a creeping stem; they are somewhat fleshy. The flowers are pink and are typical of the family, appearing during June to August.

A still further progenitor of a cultivated plant is the sea or wild beet (*Beta maritama*). It grows on the seashore, one to three feet high. The leaves are large and glossy, the ground-level (radical) ones being broad and spear-shaped, those growing up on the stems (cauline) being smaller and lance-shaped. The insignificant green flowers appear on long spikes during June to October.

During June to August, the submerged grass wracks or eel-grasses are in bloom,

The month of July sees the beginning of flowering of several outstanding seashore plants.

On maritime cliffs and rocks the tall, handsome tree-mallow grows, though it is not very common. It is a member of the mallow family (Malvaceae); but it is more nearly related to the ornamental garden plant *Lavatera* than to the mallows themselves (*Malva* species). It is *Lavatara arborea.* It grows six to eight feet high. The pale rose-purple flowers appear during July to October (Fig. 5.11).

On dunes, especially in the south-west, the bushy tamarisk (*Tamarix gallica*) blooms during July to September. This plant is native to Europe and south-east Asia where the plant assumes the habit of a tree; but in Britain it is never anything but of bushy habit. There are several different species of *Tamarix*, and several different varieties of *Tamarix gallica*, including *Tamarix gallica* var, *mannifera* of Egypt to Afghanistan, which exudes a white, honey-like secretion—the manna of the Bedouins, but not of the Israelites of the Bible. Tamarisk bears tufted branches covered with blue-green leaves, which are so reduced in size that the evaporating surfaces are cut down to a minimum—a valuable asset to a plant exposed to high winds. The plant becomes very conspicuous during July to September, when the small flowers appear to long, rose-pink spikes so closely arranged as to give a plumed effect.

The rice or cord grass, *Spartina townsendii*, begins flowering during July.

Yet another progenitor of a cultivated plant is to be found, though not easily, in salt marshes. This is the wild celery (*Apium graveolens*), and it blooms during July and August. The greenish-white flowers are borne in umbels. The staks of the leaves are not fleshy as those of the cultivated forms are.

On the seashore some particularly interesting plants begin blooming during July. One is the yellow horned poppy (*Glacium flavum*) of the poppy family (Papaveraceae). (There is also a much rarer scarlet horned poppy, *Glacium corniculatum*). The leaves of the plant are glaucous, due to a covering of wax which reduces evaporation. The large yellow flowers appear during July to September; but it is the fruit which is most interesting in that, unlike that of the common red poppy, it takes the form a horn-like pod six to twelve inches long. Inside are two longitudinal chambers containing seeds.

The strangest of all umbelliferous plants (Umbelliferae) is the sea holly (*Eryngium maritimum*), which grows on the seashore and blooms during July and August. The plant attains a height of one to two feet and bears curious leaves. The radicals leaves are round, but tough and spiny; the cauline leaves are lobed. and also have tough, spiny margins. These leaves, too, have a waxy bloom. The pale blue flowers appear in heads which look more like those of teasel than of an umbellifer, yet they really are umbels in which the separate flower-stalks have been suppressed. The roots are long and fleshy, and at one time they were candied as a sweetmeat—the 'kissing comfits' of Elizabethan days.

Sea lavender (*Limonium vulgare*) grows on the seashore. It is no relative of the garden lavender, but is closely related to thrift and more closely to garden *Statice.* All leaves are radical, being narrow at the base then broadening towards the distal end and suddenly ending in a pointed apex. The inflorescences of purple flowers appear during July and August. The flower stalks are repeatedly branched, and these bear panicles of small flowers, all of them arranged along the upper surfaces only of the branches.

In August, glasswort (*Salicornia herbacea*) begins blooming, and the insignificant green flowers appear from then until September.

The only indigenous member of the genus *Aster* (Family Compositae—a genus to which Michaelmas daisies but not cultivated asters belong, is the sea aster or starwort (*Aster tripolium*). This is an inhabitant of salt marshes and marine cliffs. Its leaves are fleshy and the flower-heads (capitula), appear during August and September. The tabular disk flowers are yellow; the fairly widely spread ligulate ray flowers are bluish-purple.

ECOLOGICAL ANATOMY

Types of Xerophytic Environment

The structural modifications common to xerophytes occur under many different environmental conditions. The most common of these is that found in deserts or semiarid places where there is a deficiency of rainfall during either a large part or all of the year. Here are found many plants, not typically xerophytic in structure, the grow only during a short rainy season and pass the dry season as seeds, or as dormant bulbs, corms, or roots beneath the surface of the ground. In such locations, with the exception, of extreme deserts where no higher plants can grow, there are also a considerable number of species which maintain stems and leaves, or only stems, above ground during the whole year. In addition to these habitats, which are characterized by actual lack of water, are those environments where water is apparently abundant, but for some reason is physiologically unavailable to the plant. Thus there is recognized a group of plants, known as *halophytes*, which grow in salt marshes or in certain types of alkaline soil that are only slightly toxic. These plants, although frequently standing in water, have elaborate structural modifications that prevent water loss, much like those found in plants of the desert flora. The water, though present, is of such high osmotic concentration that it cannot be readily absorbed by the plant; a somewhat similar condition is found in the peat bogs of the colder temperate and the subarctic regions where the water is not absorbed, not because its concentration is osmotically too high, but probably because of a toxic effect upon the roots of the plant which hinders their development or because of the very low temperature of the soil.

Another set of physiologically xerophytic conditions is that found in regions where there is no actual lack of water, but which are cold for a part of the year. Here low temperatures greatly cut down the rate of absorption by the roots and reduce conduction generally, so that even if transpiration is also reduced, there is a physiological shortage of water. This situation is more extreme where the soil freezes to a considerable depth. Plants that hold their leaves under such conditions—for example, the many species of needle-leaved gymnosperms which are generally distributed in the temperate and sudarctic zones—show typical xerophytic structure.

Exposure to persistent winds of high velocity and to intense light and heat also produce xerophytic conditions. Many xerophytic situations are the result of a combination of the environmental factors described above; in a desert situation, lack of moisture, both in soil and atmosphere, intense light and heat, and winds of high velocity may be operative.

Xerophytes

Xerophytes differ from mesophytes both physiologically and stracturally. In some plants the normal mesophytic organs and tissues are not changed in structure, but have become physiologically more effective, for example, plants growing in dry situations, such as the olive tree, develop an extraordinarily large and deeply penetrating root system capable of efficient absorption. Other examples of this kind of modification are those salt-marsh plants that have a higher osmotic concentration within the plant than is found in common mesophytes; this concentration makes possible the absorption of water from the salt-marsh soil. With this high osmotic concentration is coupled the ability of these plant cells to resist to toxic action of the salt solution, which would undoubtedly be injurious to many other plants with normal osmotic concentration. Along with physiological, are also commonly found structural modifications that reduce the loss of absorbed water. Some of the specialized structures are highly complex and may involve nearly all of the tissues of the plant.

Lignification and Cultinization. Heavy cuticularization and

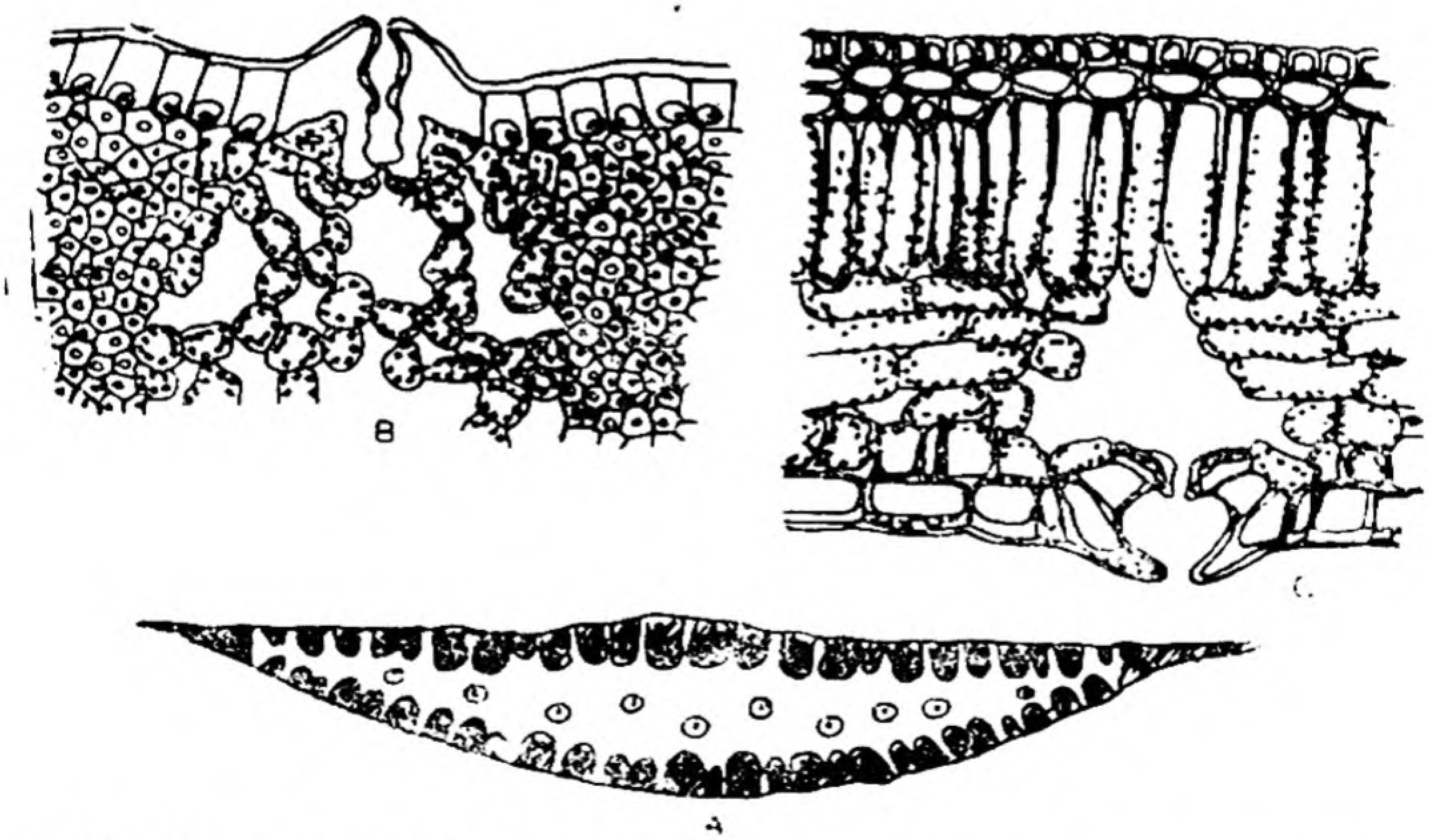


FIG. 5.12. Structure of xerophytic leaves. A diagram of cross section, Dasylirion serratifolium, sclerenchyma singly cross-hatched, vascular tissue doubly cross-hatched; B. detail of small marginal portion; C, cross section, Cycas, showing lignified hypodermis and partly lignified palisade tissue.

extreme cutinization of the epidermis and even of subepidermal cells are common in xerophytes. All gradations are found in the thickness of the cuticle from a thickness only slightly greater than normal, like that of plants of semixerophytic habitats, to the elaborate thickenings of extreme xerophytes in which the cuticle may be as thick as, or thicker than, the diameter of the epidermal cells. In addition to increased thickness of the cuticle, the walls of the epidermal cells themselves are frequently cutinized, and sometimes also those of the underlying cells. Along with well-developed cutinized layers there are frequently found different degrees of lignification of the epidermal and subepidermal cells. In some organs, for example, the leaves of *Cycas*, lignification may extend even to parts of the palisade parenchyma cells (Fig. 5.12C). Similar to cuticularization is the formation of wax as a covering on the epidermis. Many plants secrete wax externally in small amounts, but certain genera, for example, *Copernicia* and *Ceyoxylon*, the sources of carnauba wax, produce this substance in quantities sufficient to be commercially valuable. Wax is sometimes produced by plants not growing under xerophytic conditions.)

In addition to a cutinized epidermis, many xerophytic plants possess one to several layers of cells immediately, beneath the epidermis that form a *hypodermis* (Figs. 5.12C 5.14B, D). Its cells are often much like the epidermal cells in structure and are sometimes derived in ontogeny from the young epidermis, but in most plants, the hypodermis of leaves is morphologically mesophyll and may be in the form of a layer of sclereids or a sheet of fibrous tissue. The hypodermis of stems belongs to the outer cortex. The hypodermis of stems and leaves may be cutinized to some extent or, more frequently, lignified. Gums and tannins are common in this layer (Fig. 5.13A).

Sclerenchyma. In addition to the hypodermis, xerophytes generally have a larger proportion of sclerenchyma in their leaf structure than is found normally in mesophytes. This tissue, as massses either of fibres or sclereids, is usually arranged in more or less regular layers between the mesophyll

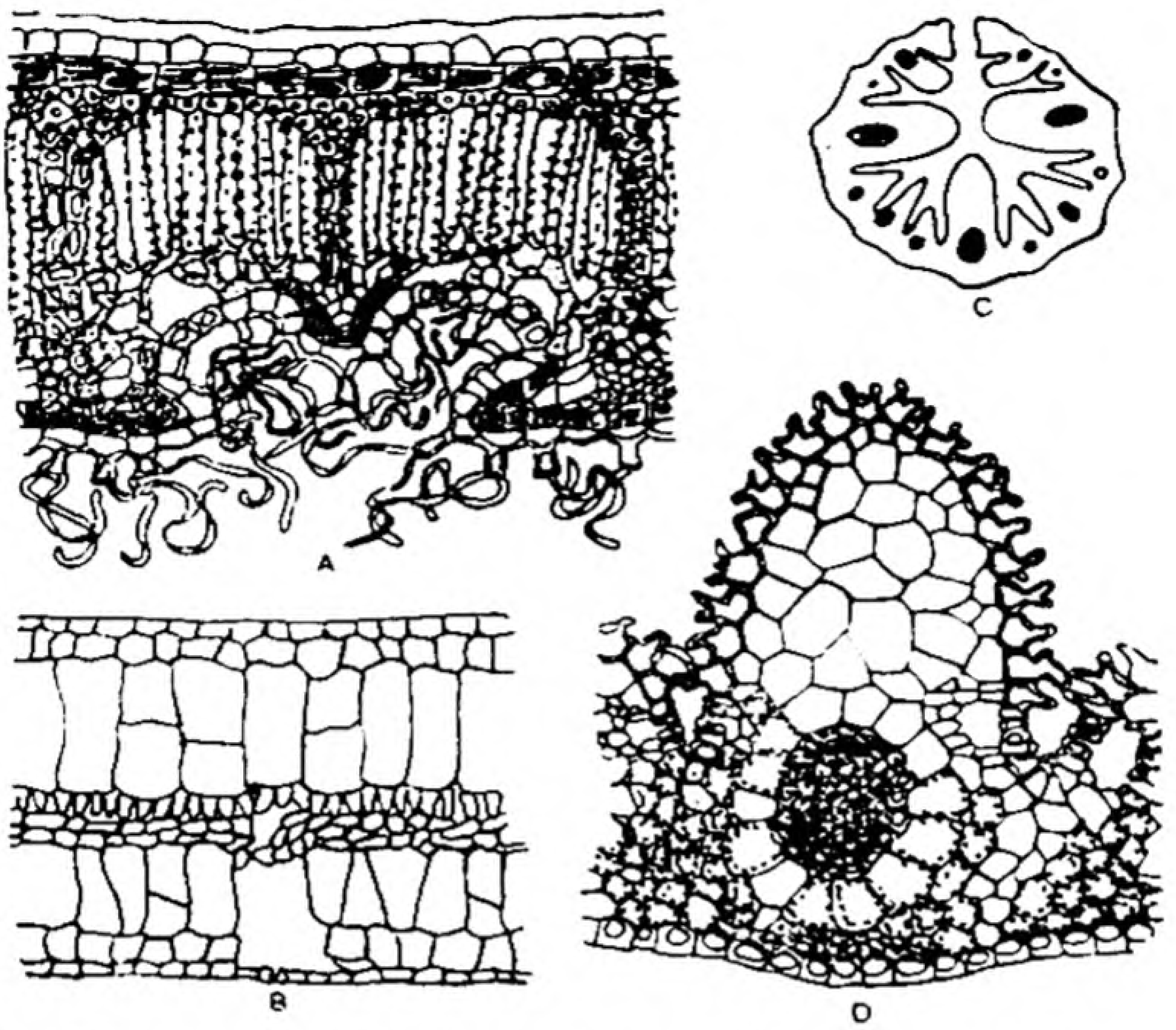


FIG. 5.13: Structure of xerophytic leaves. A, a sclerophyllous type, Banksia; B, a malacophyllous type, Begonia; C, D, a rolling, type, Spartina. A, an extreme type with very thick cuticle, outer hypodermal layer filled with mucilage and tannin, mesophyll pockets enclosed in sclerenchyma, stomata in hair-filled pockets, spongy parenchyma sparse; B, mesophyll thin, protected by layers of mucilage-containing cells, stomata unprotected; C, diagram of cross section of leaf in rolled condition; D, detail of portion of same; stomata in furrows nearly closed by interlocking epidermal cells.

of the leaf and the epidermis of hypodermis. In some plants, as in *Banksia* (Fig. 5.13A), there is a continuous, rather thin sheet of sclerenchyma between the hypodermis and the mesophyll. In other forms, for example, in *Dasylirion* (Fig. 5.15 A, B), there are heavy, parallel strands of fibres below the epidermis. These strands cover the mesophyll except for small openings leading from the stomata to the interior of the leaf. Such sclerenchyma layers prevent water loss as well as aid in the support of the organ, and may also act as a partial screen against intense light. Xerophytes that have increased cutinization and sclerification of the leaves are commonly called *sclerophyllous*.

Hairs. The modifications so far described prevent water loss from the plant by the formation of layers which are themselves more or less impervious to water. Moreover, the cutting down of the circulation of air the leaf surface prevents rapid evaporation through the stomata. In many plants, especially those of alpine regions exposed to strong winds, a covering of matted epidermal hairs on the under side of the leaves, or wherever stomata are abundant prevents water loss. Hairs may also be abundant over the entire aerial part of the plant. Hairs form dead-air spaces next to the epidermis where air remains at a relative humidity approaching that on the inside of the leaf. Xerophytes that have a hairy covering on the leaves and stems are known as *trichophyllous*.

Rolling of Leaves. The leaves of some xerophytes, of which the xerophytic grasses are the outstanding example, roll tightly under dry conditions. In such leaves the stomata are located on the upper or ventral surface only, so that when the edges of the leaf roll inward until the edges touch or overlap, the stomata are effectively shut away from the outside air. An extreme example of this kind is seen in *Spartina*, a salt-marsh grass, in which the tight upward folding of the leaf and also the sheltered location of the stomata in furrows (Fig. 5.13C, D) greatly reduce air movement over stomatal areas. In the leavs of many grasses special motor cells on the upper surface form a rolling device. In the xerophytic grasses, these cells are particularly well developed. The linear leaves of other plants, for example, species of *Dasylirion*, and those of some broad-leaved genera, such as *Rhododendron*, have leaves that fold or roll when the water supply is deficient.

Stomatal structure. Stomatal openings are essential for the intake of carbon dioxide and oxygen and possibly for the passage inward and outward of other gases. When the stomata are open, water escapes even when water loss is harmful to the plant as a whole. For this reason the reduction of transpiration is of the utmost importance in xerophytes, and reduction in number of stomata—either by reduction of leaf surface or of stomatal number per unit area—and elaborate modification of the structure of the stomatal apparatus both reduce transpiration. Water loss in xerophytes is reduced not only by hairy coverings and by the rolling or folding of leaves, but also by the position of the stomata sunken below the level of the other epidermal cells. In these stomata the accessory cells may be of such shape and arrangement that they form one or more outer chambers connected by narrow openings with the stoma itself. In this way the opening between the guard cells is shut off from the outside air with its low humidity. Stomata

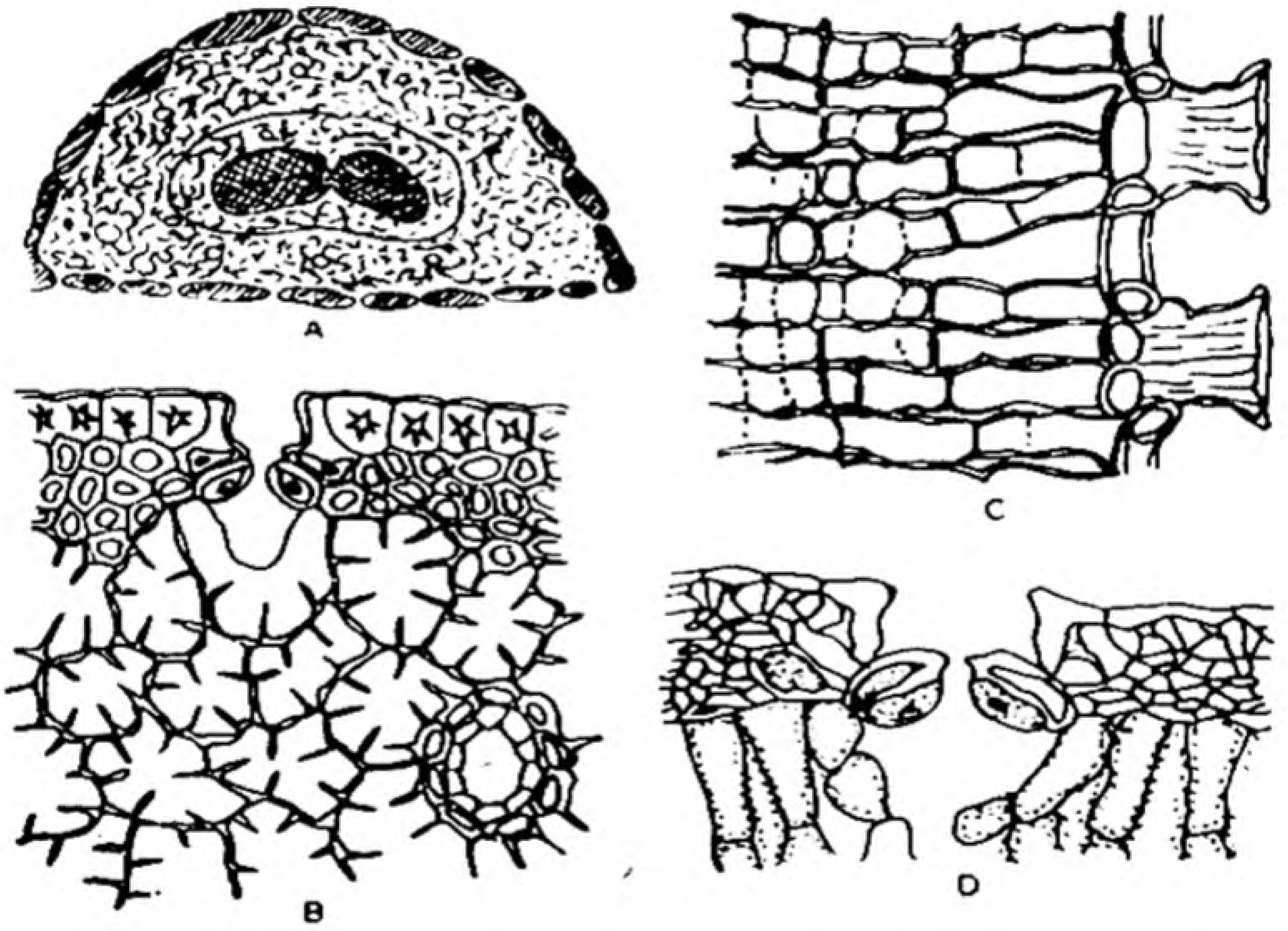


FIG. 5.14: Structure of mibrophyllous xerophytes. A, B, C, leaf Pinus nigra, diagram of cross section, detail of small part of same, and of small part of longitudinal section through stomatal furrow, respectively. A, the two vascular bundles, doubly cross-hatched, surrounded by parenchyama sheath and endodermis, the mesophyll with resin canals, protected by external sclerenchyma, singly cross-hatched, broken by stomatal furrows; B, C, the mesophyll uniform, dense, with infolded cell walls; D, cross section through stomatal region in stem, Equisetum hyemale, showing cortical photosynthetic tissue, sunken stoma, heavily silicified epidermis and hypodermis.

of this type are common in extreme xerophytes, for example, in *Pinus* (Fig. 5.14B, C), *Equisetum* (Fig. 5.14D), *Cycas* (Fig. 5.12C), and *Leptocarpus*. The walls of the accessory cells of such stomata are ordinarily very thick and heavily lignified or cutinized, as are frequently also parts of the walls of the guard cells. Sunken stomata as in *Casuarina* and *Banksia* (Fig. 5.12A), may have the further protection of hairs.

Reduced leaf surface. *Microphyllous* plants form one of the larger groups of xerophytes. In this group the reduction of the leaf surface partly prevents water loss because the total exposed surface of the plant body is relatively small as compared with that of normal mesophytes. Abundant illustrations of this type of xerophyte occur in all groups of plants, for example, *Equisetum*, *Pinus*, *Casuarina*, *Asparagus*, the cacti, *Polygonella*. In these forms the leaves, if normal in function, are very small, often they are wanting in the mature plant or persist as small scales or bracts which for the most part do not function as leaves. In some genera, for example, *Equisetum*, *Leptocarpus*, and *Polygonella*, photosynthesis takes place in the stem where photosynthetic tissues are well developed. The reduction of leaf surface is commonly accompanied by increased sclerenchyma, sunken stomata, and water storage.

Needle leaves of the gymnosperms. The needle leaves of the gymnosperms are in important microphyllous type. Many species of *Pinus*, *Picea*, and other coniferous genera are exposed to xerophytic conditions only during the winter when low soil and air temperatures prevent water absorption and conduction and do not prevent water loss from the leaves. In the leaves of these plants, in addition to reduced leaf surface there are heavy eutinization and sunken stomata. A characteristic needle leaf is the leaf of *Pinus* (Fig. 5.14 A-C). In this leaf the vascular tissue consists of two collateral bundles each made up of about equal amounts of xylem and phloem, which are largely secondary in origin. Immediately surrounding the vascular tissues is a zone of so-called "transfusion" tissue limited by a well-defined endodermis. Some of the cells of the transfusion tissue, which is in the pericyclic position, show bordered pits even though the tissue has otherwise the appearance of parenchyma. Exterior to the endodermis is the photosynthetic tissue, which is made up of a special type of mesophyll cells. In these cells the inward projections of the wall greatly increase the wall surface along which the chloroplasts are distributed. The outer walls of the epidermal cells are cutinized, and the cutinization extends along the middle lamellae between and around the cells. The wall is thickened to such an extent that the lumen of the cells is nearly occluded. Beneath the epidermis is a well-developed hypodermis several layers thick, consisting of elongate sclerenchyma cells extending parallel with the long axis of the leaf. The stomata are somewhat sunken and arranged in definite longitudinal rows. Their structure as seen is transverse and longitudinal sections of the leaf in shown in (Fig. 5.14 B, C). The needle leaves of other gymnosperms, though differing from those of *Pinus* are for the most part similar in general features.

Fleshy Xerophytes. A fourth large group of xerophytes, those possessing fleshy leaves or stems, are described as *malacophyllous.* In these plants, tissues that store water and mucilaginous substances are prominent. In leaves these tissues are located beneath the upper or the lower epidermis, upon both sides of the leaf, or, in extreme types, on both sides of the leaf and in the center. The storage cells are usually large and often thin-walled, as in *Begonia* (Fig. 5.13 B). Frequently, the walls are reinforced in a manner that prevents collapse when turgor is reduced. Such storage tissue apparently may actually serve as a source of reserve water during drought, or may screen the underlying tissues from excessive light. In some species the thickened leaves are terete with the vascular bundles arranged in a stele-liks cylinder. The mesophyll, consisting frequently of a large proportion of palisade tissue, is more compact than in mesophytes, and the amount of photosynthetic tissues is greater relative to the amount of leaf surface exposed. The leaf surface is, therefore, proportionately reduced. Water-storage tissue is abundant in many microphyllous xerophytes, especially the cacti and plants of similar habit, and some salt-marsh genera, such as *Salicornia*.

Epiphytes. The structure of epiphytes varies with the environment. Many are xerophytic in their general structure, for example, *Tillandsia* and many orchids; the former has a hairy covering and the latter heavily cutinized epidermis and relatively small leaf surface. The root system of epiphytes may consist, in part, of holdfasts, which anchor the plant; in part, of absorbing roots, which are in contact with the substratum, and in some genera, of aerial roots. Some epiphytes live in habitats that are always moist and have no xerophytic structure.

Hydrophytes. The number of species of vascular plants that grow submersed in water, or floating, is much smaller than the number of xerophytes and they show much less variety in structure than xerophytes; the uniformity of aquatic environments is in contrast with the variety of xerophytic environments. The factors affecting aquatic plants are chiefly those of temperature, osmotic concentration, and toxicity—the last two dependent upon the amount and the nature of the substances in solution. The structural characteristics in aquatic plants are mainly those of reduction of the protecting, supporting, and conducting tissues, and the presence of air chambers. (The effectiveness of xerophytic structure against drying out can be seen when a xerophyte, such as *Sedum*, and a hydrophyte, such as *Potamogeton*. are exposed to drying conditions; the former will remain alive for many days, whereas the latter will dry out in a very few hours.)

Epidermis in hydrophytes. The difference in the structure and function of the epidermis in hydrophytes as compared with that of plants in an aerial habitat is outstanding. In aquatics, the epidermis is not protective but absorbs gases and nutrients directly from the water. This layer in the typical hydrophyte has an extremely thin cuticle, and the thin cellulose walls permit ready absorption from the surrounding water. Commonly in

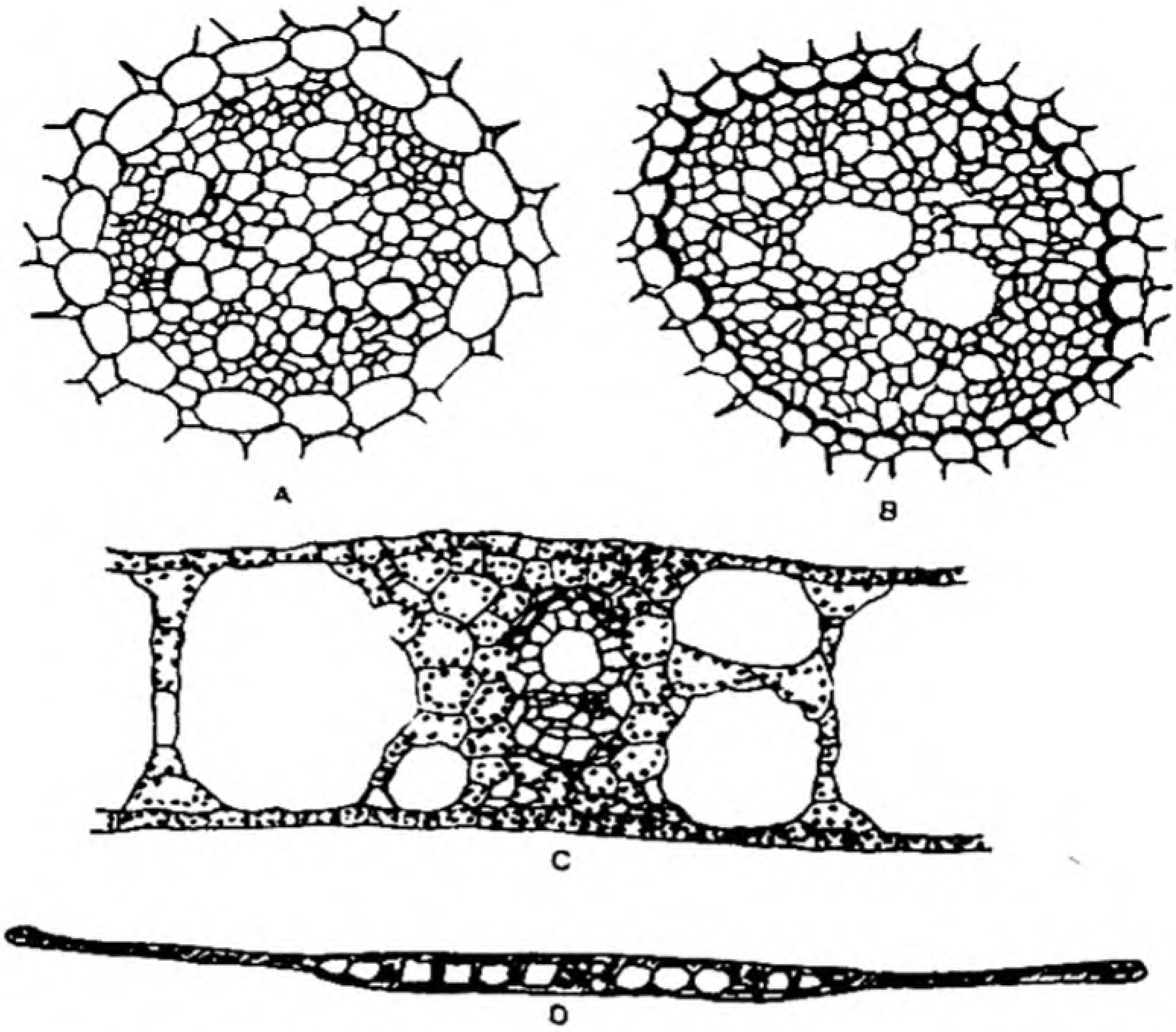


FIG. 5.15: Structure of hydrophytes. A, B, cross section of stele of stems, Elatine Alsinastrum and Potamogeton pectinatus, respectively: A, xylem cylinber greatly reduced, phloem cylinder complete; B, xylem lacking, its position occupied by lacunae, phloem well developed and surrounding the lacunae, inner wall of endodermis much thickened. C, D, cross sections of submersed leaf of Potamogeton epihydrus: D, diagram, showing air chambers and bundles; C, detail of central portion of D, showing reduced bundle with the xylem lacking, sclerenchyma reduced, all other cells thin-walled, epidermis with chloroplasts, stomata lacking. (A, B, after Schenck.)

aquatics, the epidermis contains chloroplasts and may thus form a considerable part of the photosynthetic tissue, especially where the leaves are very thin. Stomata are wanting in submersed hydrophytes (though sometimes vestigial); the gaseous interchange takes place directly through the cell walls. The floating leaves of aquatic plants have abundant stomata on the upper surface.

Dissected leaves. In many species of aquatic plants the submersed leaves are finely divided so that there is, proportionately, a much increased leaf surface in contact with the water. The slender terete segments of such leaves permit close contact between photosynthetic tissues and the water. Such genera as *Myriophyllum* and *Utricularia* are examples of aquatics with divided leaves of this type. Some species with divided, submersed leaves have floating or aerial leaves which are entire, toothed, or lobed.

Air chambers. Chambers and passages filled with gases are common in the leaves and stems of submersed plants. Water-storage cavities are structurally single cells, but air chambers are large, usually regular, intercellular spaces extending through the leaf and often for long distances through the stem. Good examples of this type of structure are found in the leaves of ***Potamogeton*** (Fig. 5.15 D. C) and ***Pontederia*** (Fig. 5.16 A). Such spaces are usually separated by partitions of photosynthetic tissue only one or two cells thick. The chambers provide a sort of internal atmosphere for the plant. In these spaces the oxygen given off in photosynthesis is apparently stored and used again in respiration, and the carbon dioxide from respiration is held and used in photosynthesis. The cross partitions of air passages, known as diaphragms, perhaps prevent flooding. The diaphragms are perforated with minute openings (Fig. 5.16 B) through which gases but not water may supposedly pass. Air chambers also give buoyancy to the organs in which they occur. Another type of specialized tissue frequent in aquatic plants that gives buoyancy to the plants parts on which it occurs is *aerenchyma.* It is characteristic of species of *decodon* and *Lythrum*, for example. Structurally, it is very delicate tissue in which thin partitions enclose air spaces (Fig. 5.16 C). Aerenchyma is phellem formed by a typical phellogen of epidermal or cortical origin. The phellem cells are small and thinwalled, and a very delicate tissue is built up as the young phellem cells mature. At regular intervals individual cells or each layer elongate greatly in the radial direction while the other cells of this layer remain small. The elongation of these cells pushes outward all the cells of the last-formed layer and forms many elongate air chambers that lie parallel with the plant axis on which they are borne. The radially elongated cells form the radial walls of the chambers and the nonelongate cells which have become separated from the adjacent tangential rows of cells, the tangential walls. In a physiological sense, the term "aerenchyma" is applied to any tissue with many large intercellular spaces. Such tissues may be morphologically a part of the cortex or pith, and hence quite distinct from the typical aerenchyma described above which is of secondary origin.

Absence of Sclerenchyma. Submersed plants usually have few or no sclerenchymatous tissues and cells; the water itself supports the plant and protects it in part from injury. Density of tissues, thick walls, and in some plants collenchyma, give

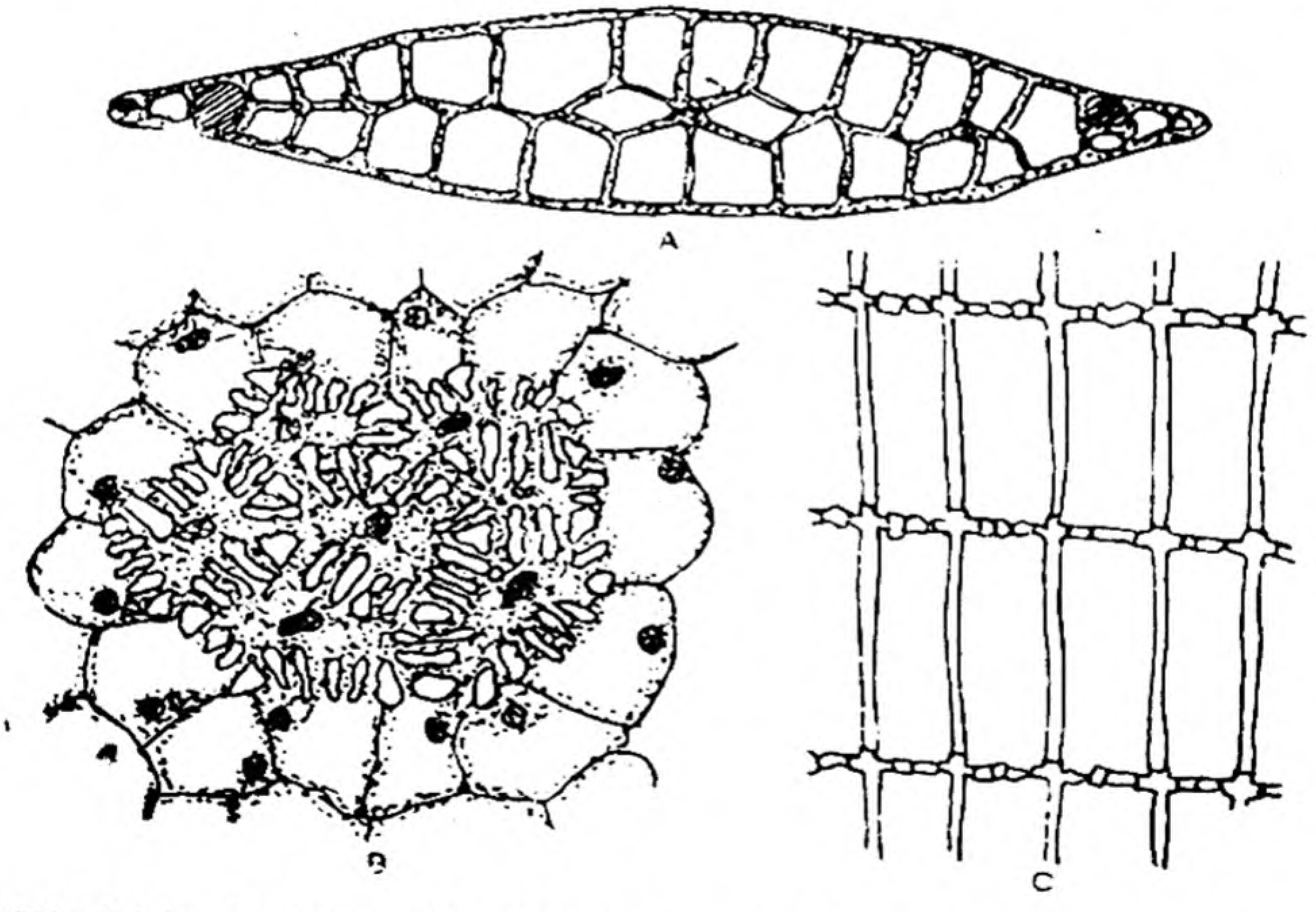


FIG. 5.16: Structure of hydrophytes. A, B, Pontederia cordata: A, cross section of submersed leaf, showing air chambers and reduced vascular bundles (doubly cross-hatched), diaphragms in air chambers singly cross-hatched. B, diaphragm in detail showing performations (unshaded). C, aerenchyma in Decodon, cross-section, showing air spaces formed by elongation of certain cells of each phellem layer.

some rigidity, but submersed plants, especially the leaves and smaller stems, are usually flaccid and collapse when removed from the water. Sclerenchyma strands occasionally occur, especially along the margins of leaves; here they apparently increase tensile strength.

Reduction of vascular and absorbing tissues. An aquatic plant is, in reality, submersed in or floating upon a nutrient solution. Those structures that in land plants absorb mineral nutrients and water from the soil and conduct these substances through the plant are greatly reduced or absent in aquatic plants. The root system is often greatly reduced, functioning chiefly as holdfasts or anchors, and a considerable part of the absorption takes place through the leaves and stems. All degrees of reduction of the root system are found. Even where reduction of roots is not extensive, root hairs are usually lacking, and the roots probably do not absorb water to any extent. In the vascular tissues, the xylem shows the greatest reduction and is many species consists of only a few elements, even in the stele and main vascular bundles (Fig. 5.15 A). Less commonly in the stele and large bundles, and frequently in the small

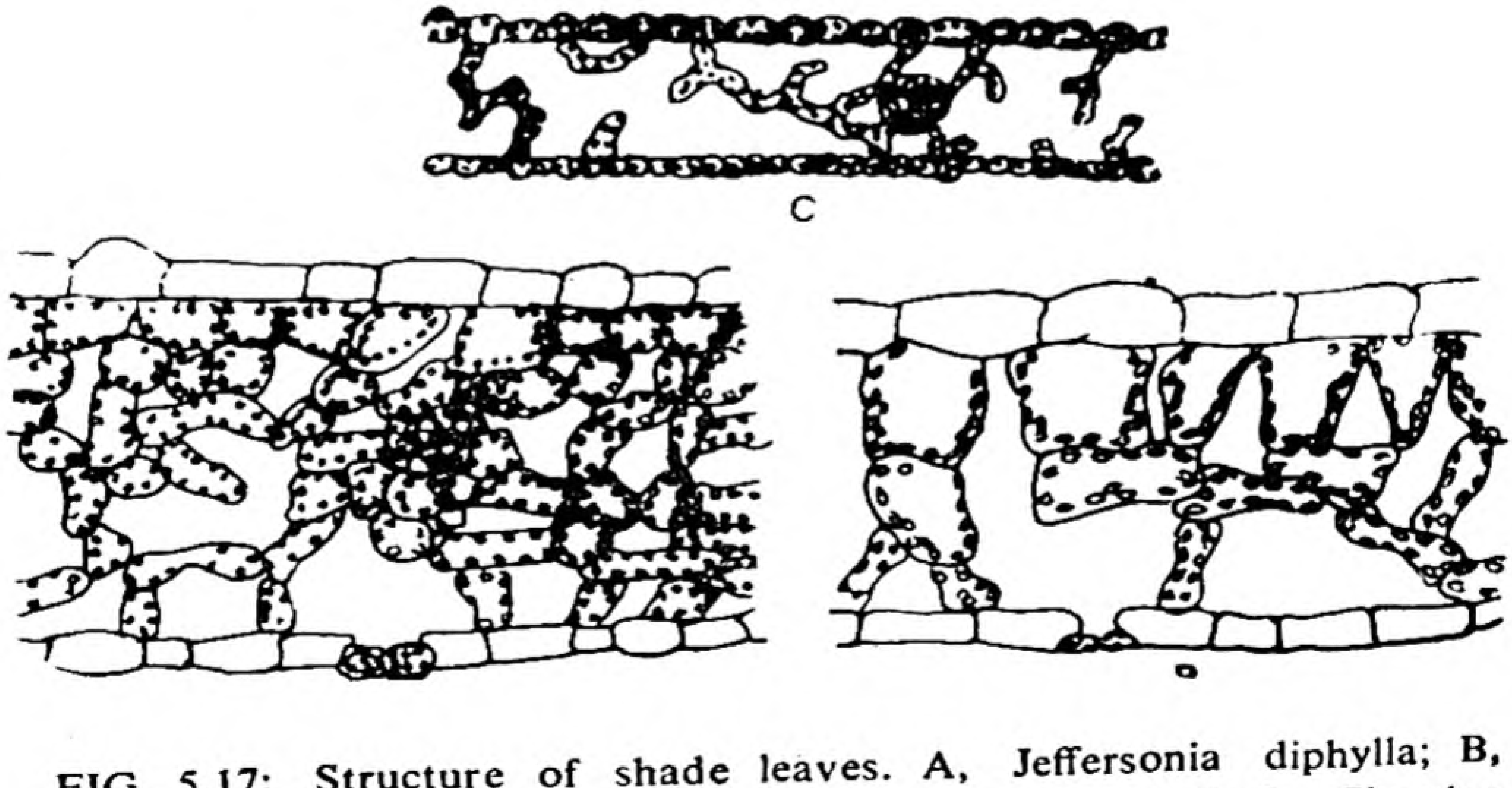


FIG. 5.17: Structure of shade leaves. A, Jeffersonia diphylla; B, Circaea alpina; C, Cruptogromma Stelleri. Showing mesophyll loose, palisade layer weak or lacking, and epidermis weakly cutinized—C, extreme form, suggesting hydrophytic condition, with chloroplasts in epidermis.

bundles, xylem elements are lacking. In these plants there is usually a well-defined xylem lacuna in the position of the xylem (Fig. 5.15 B, C). Such spaces resemble typical air-chambers. The phloem of aquatics, though reduced in amount as compared with that of mesophytes, is in most species fairly well developed as compared with the xylem. It resembles the phloem of reduced herbaceous plants, in that the sieve tubes are smaller than those of the woody plants. These reduced bundles are illustrated in the leaf of *Potamogeton* (Fig. 5.15 C, D). In aquatics an endodermis is commonly present, though often weakly developed.

Shade Leaves. The leaves of deeply shaded habitats have weakly developed palisade layers. This type of leaf structure is found in many mesophytic plants such as low-growing woodland plants, for example, *Jeffersonia* (Fig. 5.17 A). The more extreme plant types in such habitats, such as *Cryptogramma* (Fig. 5.17 C), a fern of moist, shaded cliffs, lack palisade layers and may possess an epidermis that contains chloroplasts and becomes a part of the photosynthetic tissue. Hydathodes, where water is exuded, are frequently found in plants growing in a saturated atmosphere.

Parasites

In parasitic, saprophytic, and insectivorous vascular plants in which the plant is dependent, wholly or in part, upon other organisms, there are prominent structural modifications. In parasitic vascular plants there are commonly no root except in the seedling stage before connection with the host is made. Such plants as *Cuscuta* and *Conopholis* are familiar examples in which, early in the life of the seedling, the parasite establishes direct connection by means of its haustoria with the conducting system of the host. In the region of this connection, the xylem and phloem of the parasite join directly with the same tissues of the host, supplying the parasite with water, mineral nutrients and elaborated plant foods. The xylem and phloem of the parasite come in direct contact with the xylem and phloem of the host through a dissolution of the outer tissues of the host by enzymes secreted by the parasite at the points of contact where haustoria are formed (Fig. 5.18 B). New secondary vascular tissues are then formed by the parasite, and these continue the union with the newly formed tissues of the host.

In true parasites, all photosynthetic tissues are greatly reduced, exist as vestigial structures, or are wanting. In such plants as *Cuscuta* and *Arceuthobium*, the leaves are reduced to scales, and chlorophyll is usually absent throughout the plant. *Cuscuta* may form a large number of host connection. Other plants, for example, *Conopholis* and *Orobanche*. make only one host connection but penetrate deeply into the root of the host. In such species the parasite may form a flowering axis only. Besides these complete parasites, which depend wholly upon the host for food and water, there are many so-called "half-parasites," which, although connected directly with the vascular tissues of the host, apparently manufacture some part of their own food supply. The mistletoes, *Viscum* and *Phoradendron*, are familiar examples. Here water and mineral nutrients are obtained entirely from the host. Carbohydrates are probably, at least in part, formed by the parasite. The vascular tissues of parasites are usually much reduced. Sclerenchyma often supplies nearly all the support of the stems.

"*Saprophytes*". The term *saprophytes*, as commonly applied to vascular plants, refers to a group of chlorophyll-lacking forms that have been said to secure their food supply from decaying organic matter, much as do many fungi. Many and probably all such plants are associated more or less closely with fungi in their undeground parts. The method by which such plants obtain food materials is apprrently bound up in some way, not well understood, with the physiologicol activities of these fungi. But clearly such plants are not strictly saprophytes—they are, to a certain extent, at least, symbiotic with or parasitic upon the lower forms, or are associated in nutrition with them. It is undesirable to call such plants "saprophytes" until their methods of nutrition are fully understood. Plants of this types are usually greatly reduced in structure—the leaves are scale-like and the stems are reduced largely to inflorescence exes. Internally, there is also great reduction and simplification. The xylem and the phloem are small in amount and the cells of these tissues often abortive, and sclerenchyma is scarece. The roots may be well developed and abundant in proportion to the aerial parts, as in *Monotropa*, or may be lacking, with rhizomes taking their place, as in *Corallorrhiza*. Where roots are present, they are usually of peculiar structure. Roots of unusual form, together with the fungus hyphae associated with them, form *mycorrhizae*.

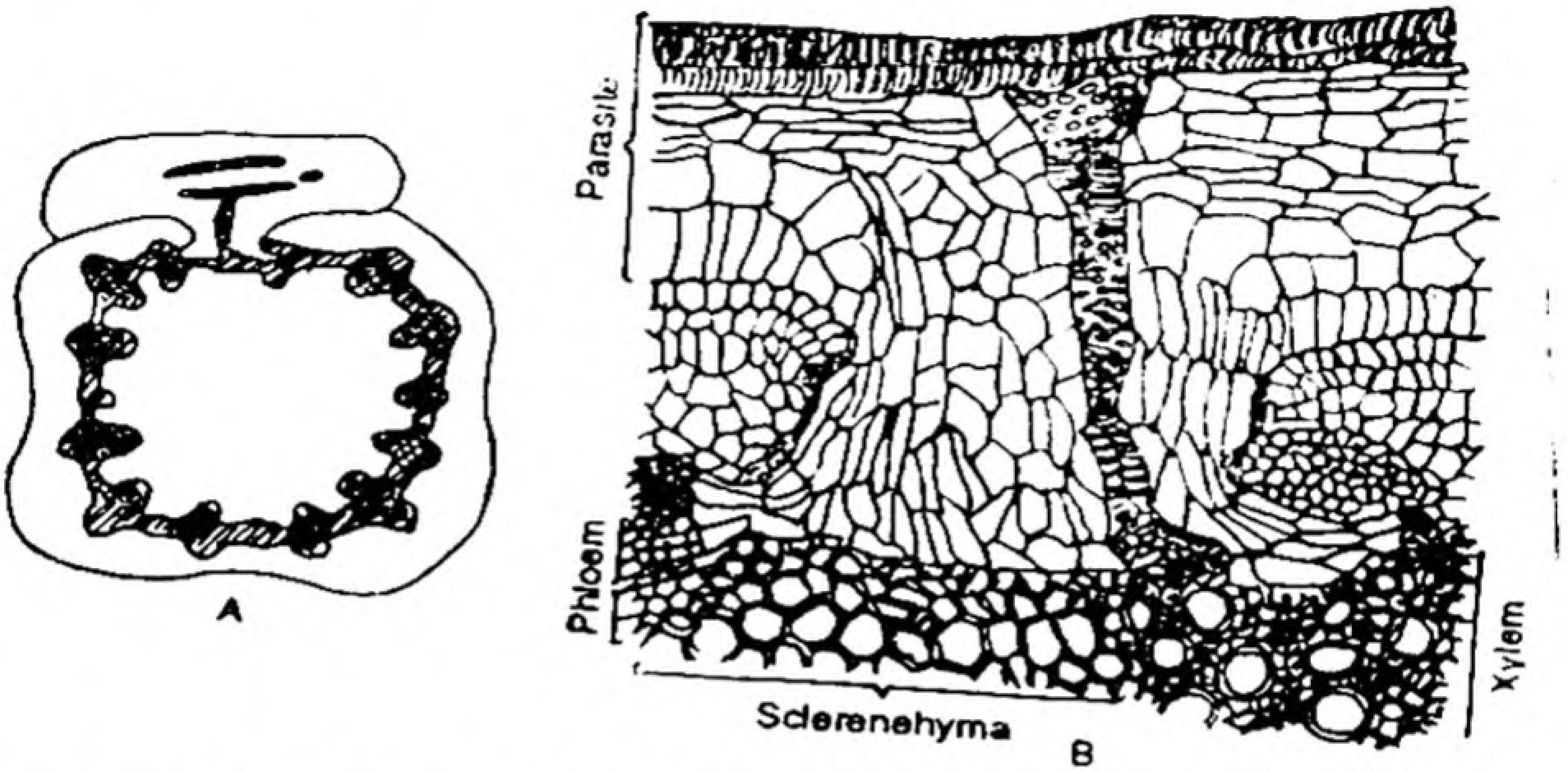


FIG. 5.18: Haustorial connection of parasite with host—Cuscuta on Bidens. A, diagram, showing cross section of stem of host, oblique section of stem of parasite, and longitudinal section of haustorium penetrating host as far as the vascular tissues; B, details of haustorium and surrounding tissues. The vascular tissues of parasite cannected with those of host.

Two types of such roots are recognized: *ectotrophic*, those in which the mycelium forms a more or less superficial layer or coating about the roots, and *endotrophic*, those in which the hyphae lie within or between the root cells themselves. In ectotrophic forms the fungus hyphae form a tissue-like weft about the root, enclosing it like a glove about a finger. The mycelium is closely appressed to the root, and some of the hyphae penetrate between and around the epidermal and subepidermal cells. In some forms there is little penetration between the outer cells, in others the cells of the epidesmis and of several outer layers of the cortex may be surrounded by hyphae. Rootlets invested by fungus hyphae in this way are of unusual structure—they have neither root cap nor root hairs, have limited growth in length, and are fleshy, with their conducting tissues greatly reduced. In endotrophic forms also, the roots may be enlarged and fleshy but there is less reduction of vascular tissues. The hyphae are usually restricted to a special part of the cortex where a definite layer of infected cells may occure. In nongreen plants the interrelationship of the two plants is obscure.

Mycorrhizae occur on many green plants also; they are known in many genera of forest trees and in many members of the health family. The relationship of the fungus and the green vascular plant is not understood but is possibly parasitism of basidiomycetous fungi upon the green plant. In some endotrophic forms, symbiosis may exist.

The above discussion is only a brief statement of some structural modifications of plants. The details of such modifications are almost infinite in number and veriety. All can be structurally interpreted by comparision with mesophytic plants.

6

Carnivorous Plants

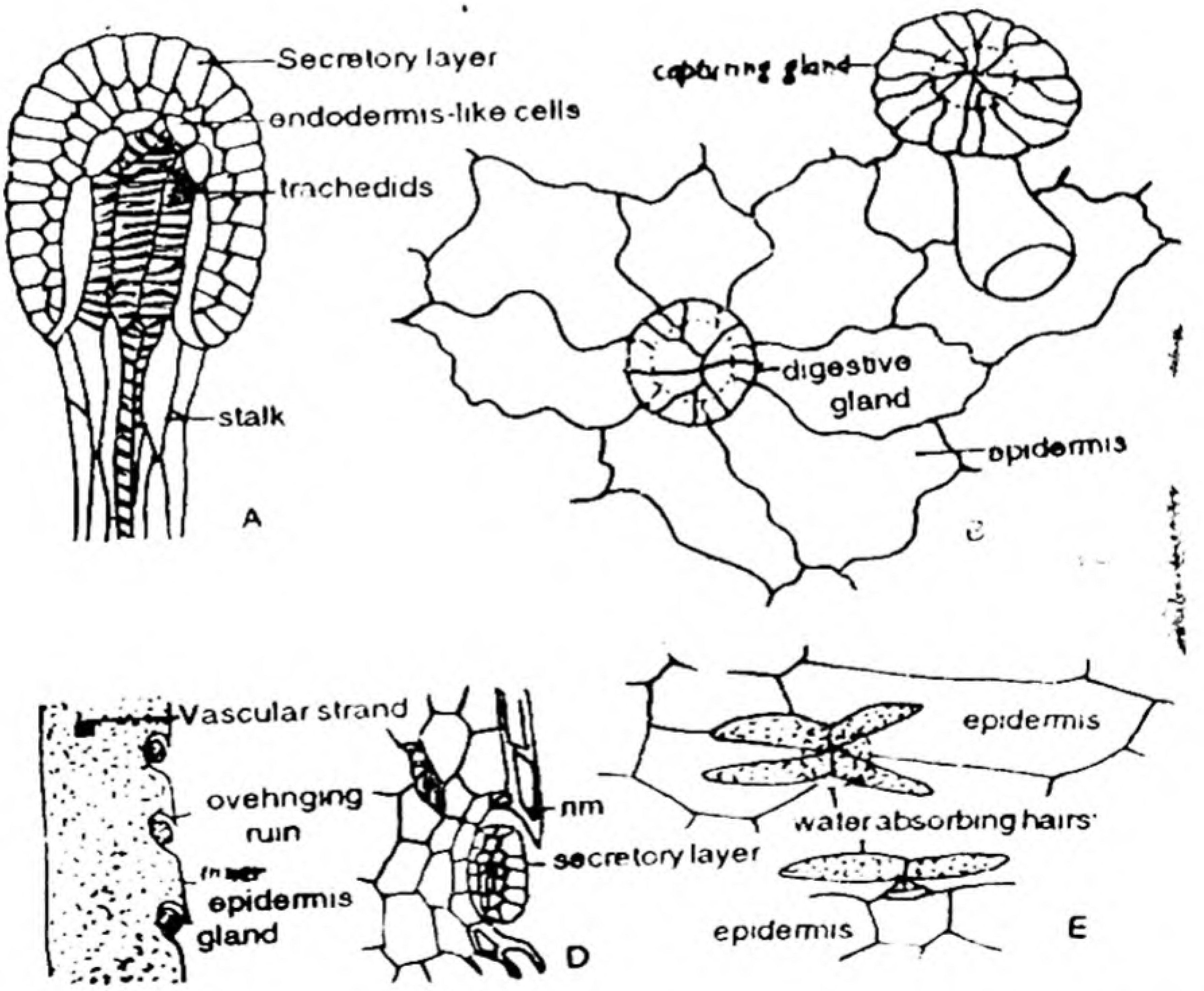
Although plants are consumed whole or in part by animals. there are very few cases where plants make direct use of animals as a source of food. Normally they only utilise animal bodies and excretions after these have been converted by bacteria into simple nitrogenous substances.

Nevertheless, there are examples of plants preying on living animals for the purpose of nutrition. They actually catch the animals, kill them, and then digest them. Animal-eating plants have caught the imagination of many a person, and are especially common in stories of travellers of days gone by. For example, for many years travellers in the unknown parts of certain tropical regions used to come home with awe-inspiring tales of a 'man-eating tree', that is, a tree which was able to trap a man if he gote too near it and then digest him. Though this myth held sway for a long time, no such tree exist. In fact, comparatively few animal-trapping plants exists, and these catch only insects and other very small animals.

Carnivorous Plants

Some piants, since they consume insects and other small animals, are called *insectivorous* or *carnivorous* plants. Although comparatively rare, the are of extreme interest in that they have all developed most wonderful methods for catching their prey.

Much work on the insectivorous plants was done, chiefly by the best method, that of field observation, by Charles Darwin, who published an account of them in 1875. Before considering any examples of carnivorous plants, it is necessary to reflect on them in general, chiefly to get rid of any exaggerated ideas that we may have of them. Some of them may be found in Great Britain. It is quite true that they trap insects and other small animals, and absorb certain digestible parts of the body of their victims. But not one carnivorous plant depends *comtpteley* on insects for its nutrition. It is possible to go even further


Secretory layer
endodermis-like cells
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A
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digestive gland
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nm
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D
epidermis
water absorbing hairs
epidermis
E

FIG. 6.1: Glands of Carnivours Plants. A, head of tentacle of Drosera; B. surface view of part of a leaf of Pinguicula; C and D, wall of the lower part of a pitcher of Nepenthes; E, the quadrified hairs on the inside of the bladder of Utricularia, in surface view (above) and in section (below) (F.W. Williams).

than that: *insects never form more than a very small fraction of the food of carnivorous plants.* There is no need to watch such a plant and see how many insects it catches in a certain length of time to learn this, for, when it is realised that all carnivorous plants possess green leaves, it can be concluded immediately that their main source of food supply is the normal one, that is, photosythesis.

Since this is the case, the question arises: why do such plants trap insects? The majority of carnivorous plants grow in very wet situations, such as swamps and wet moors. In such regions the waterlogged condition interferes with the bacterial breakdown of organic matter and so leads to the accommulation of peat. This is deficient in mineral salts and particularly in nitrates. A few carnivorous plants grow as epiphytes in wet tropical forests and these, too, will tend to suffer from nitrogen deficiency. Now insects and other small animals which these plants trap have a high protein content in their bodies. Thus, by digesting them, carnivorous plants obtain an extra supply of nitrogen, to make up for the deficiency of teat element in such habitats.

Thouph carnivorous plants actually can do without such animal food, there is little doubt that the nutrition obtained by them is valuable.

CARNIVOROUS PLANTS OF TEMPERATE REGIONS

A few species of plants of insectivorous habit are to be found growing wild in Great Britain.

One rather common carnivorous plant is the *butterwort* (*Pinguicula vulgaris*).

This is a perennial herb with leaves 1 to 3 inches long, arranged in rosette fassion. The upper surface of the leaves is covered with a pale yellow, sticky substance which looks like a thin layer of better; hence the common name and the generic name, the latter being derived from the Latin *pinguis*, oily. An unwary insect alighting on this sticky surface gets caught like a fly on a fly-paper. The margins of the leaf are slighly incurved, and when an insect is caught, they curve over still further, thus gripping the insect.

On the surface of the leaves are certain microscopic structures called *glands*. These are of two kinds (Fig. 6.1 B). Stalked glands, looking like miniature mushrooms, secrete a sticky mucilage and are the *capturing glands*. Sessile glands, borne at the level of the leaf surface, are *digestlve glands*. When an insect has been captured, these produce an abundant acid secretion which contains proteolytic enzymes. This proteins of the insect body are thus broken down into simpler nitrogenous substances which are then absorbed by the surface of the leaf. After absorption the leaf unrolls, and in due course the insect remains are either blown or washed away.

This plant is quite common in Great Britain, especially on the Somersetshire Plain, and in Yorshire and Scotland. It is very widely distributed in the north temperate zone, and is common in certain parts of Canada and the United States.

There are a few rarer species of butterwort, for example, the Irish butterwort (*P. grandiflora*), Alpine butterwort (*P. alpina*) and Western butterwort (*P. lusitanic*).

Butterwort flowers appear during May to July. They are borne on erect, thick stalks and are purple in colour, tow-lipped in form with a backwardly projecting spur.

Another British insectivorous plant, but one with a more complicated trapping mechanism, is the *round-leaved sundew* (*Drosera rotundifolia*). The leaves, all of which are radical, again supply the trapping mechanism. They are green with patches of red on them, and possess long petioles. The laminae are round or oval according to the species. The leaves are again arranged in rosette fassion. The flowsrs are white, and are arranged in rather long, upright inflorescences.

The edges and upper surface of the leaf of the sundew are covered with long, hair-like outgrowths called *tentacles* (Fig. 6.1 A). There are about 200 tentacles on one lamina. Each tentacle ends in a club-shaped glandular head which secretes a sticky substances looking like dew; hence the common name and the generic name which is derived from the Greek *drosos*, dew. When the insect alights on the leaf, it is thus caught by the sticky tentacles. Then there is a general bending of all the tentacles towards the centre of the lamina, with the result that the insect is caught and pressed firmly against the leaf surface by the tentacles (Fig. 6.2). The ends of the tentacles then secrete a protein-digesting enzyme, and thus the insect is digested and the products are finally absorbed. After the process is finished, the leaf attains its normal form again and the indigestible parts of the insect are exposed and blown away by the wind. The characteristics nastic movements of the tentacles are stimulated both by contact and by chemical substances such as ammonium salts and proteins. They are brought abcut by increased growth of the lower side of the tentacles near their base, Subsequent unfolding results from growth of the upper surface of the tentacles.

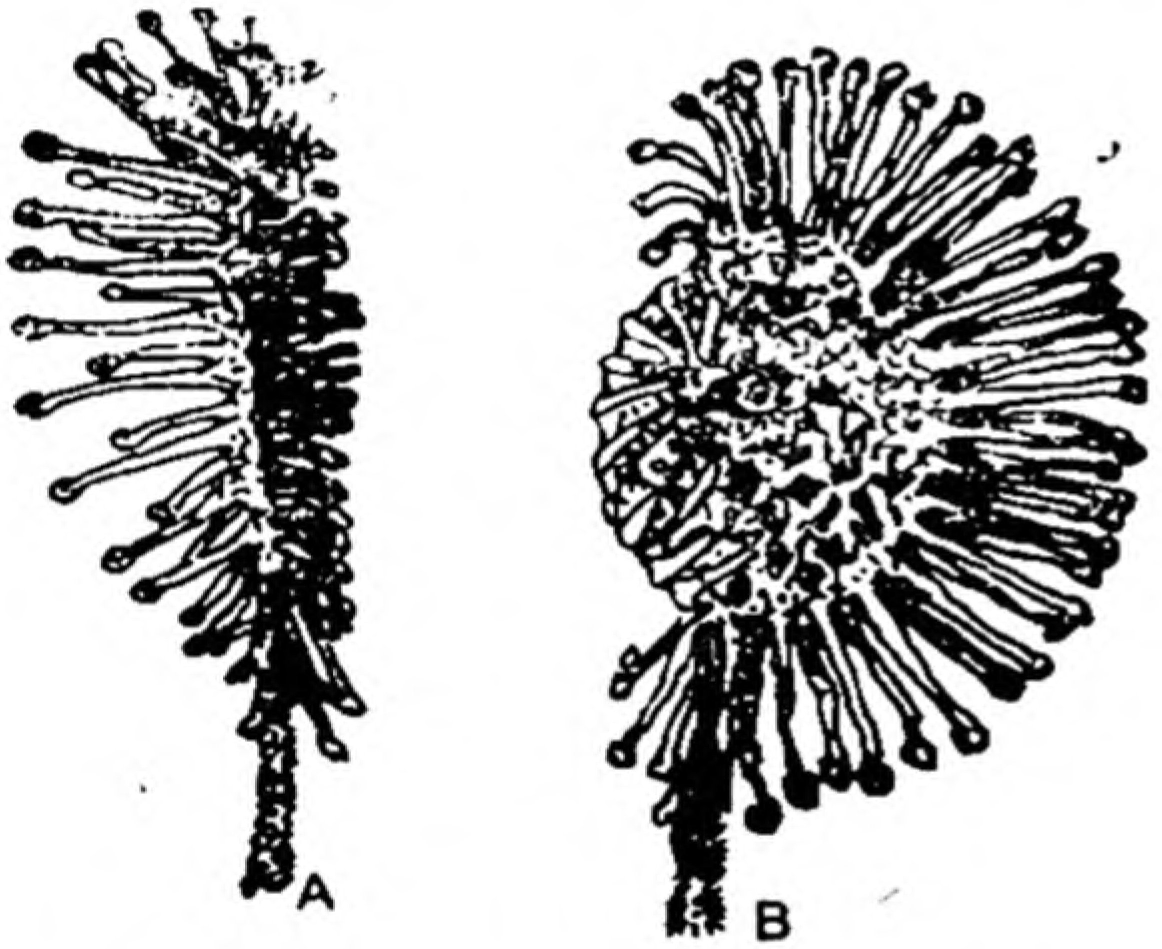

FIG. 6.2: Leaf of Sandew. Leaf, side view; right, front view after being touched with a pencil.

The round-leaved sundew (*Drosera rotundifolia*) is common in the swampy region of Great Britain, favouring acid bogs and the moist hallows of sandy heaths. There are two other species, the long-leaved sundew (*D. anglica*) which is rare in the south of England, and the lesser long-leaved or the narrow-leaved sundew (*D. intermedia*, formerly *longifolia*) which is fairly well distributed but not so common as the round-leaved. Sundews are also common in North America, and are abundant in Australia.

Sundew flowers are borne on erect, thin, stalks varying from two to eight inches in height. The flowers appear during June to August in circinate cymes and are white in colour.

An even more specialised method for trapping small animals is shown in another well-known insectivorous perennial plant called the *bladderwort* (*Utricularia vulgaris*), a brakish-water plant which grows in ditches and ponds in certan parts of Great Britain.

The leaves of the bladderwort are very finely divided, as is the case in many examples of submerged leaves. Certain of these leaf segments become much modified into bladder-like structures, thus giving the plant its familiar name (Fig. 6.3) Each bladder has one opening which is protected by a valve. The valve will open inwards, but will not open outwards. At one time it was considered that small aquatic animals forced their way into the bladder (probably seeking sanctuary from enemies). This, however, is not true. They are actually captured by the bladder. The organism enters the vestibule of the bladder and may stimulate the valve to open by touching sensitive hairs growing on the outer surface of the valve itself. The walls of the bladder, hitherto compressed, now expand and draw water in, thus sucking in the prey. The valve now closes again so that egress of the prey is prevented. The whole of the 'gulping' action resulting in the capture of the prey takes only about $\frac{1}{30}$ of a second. Water-fleas and minute insect larvae are the usual prey. These swim about in the bladder for a time and then die. There is no evidence that digestive

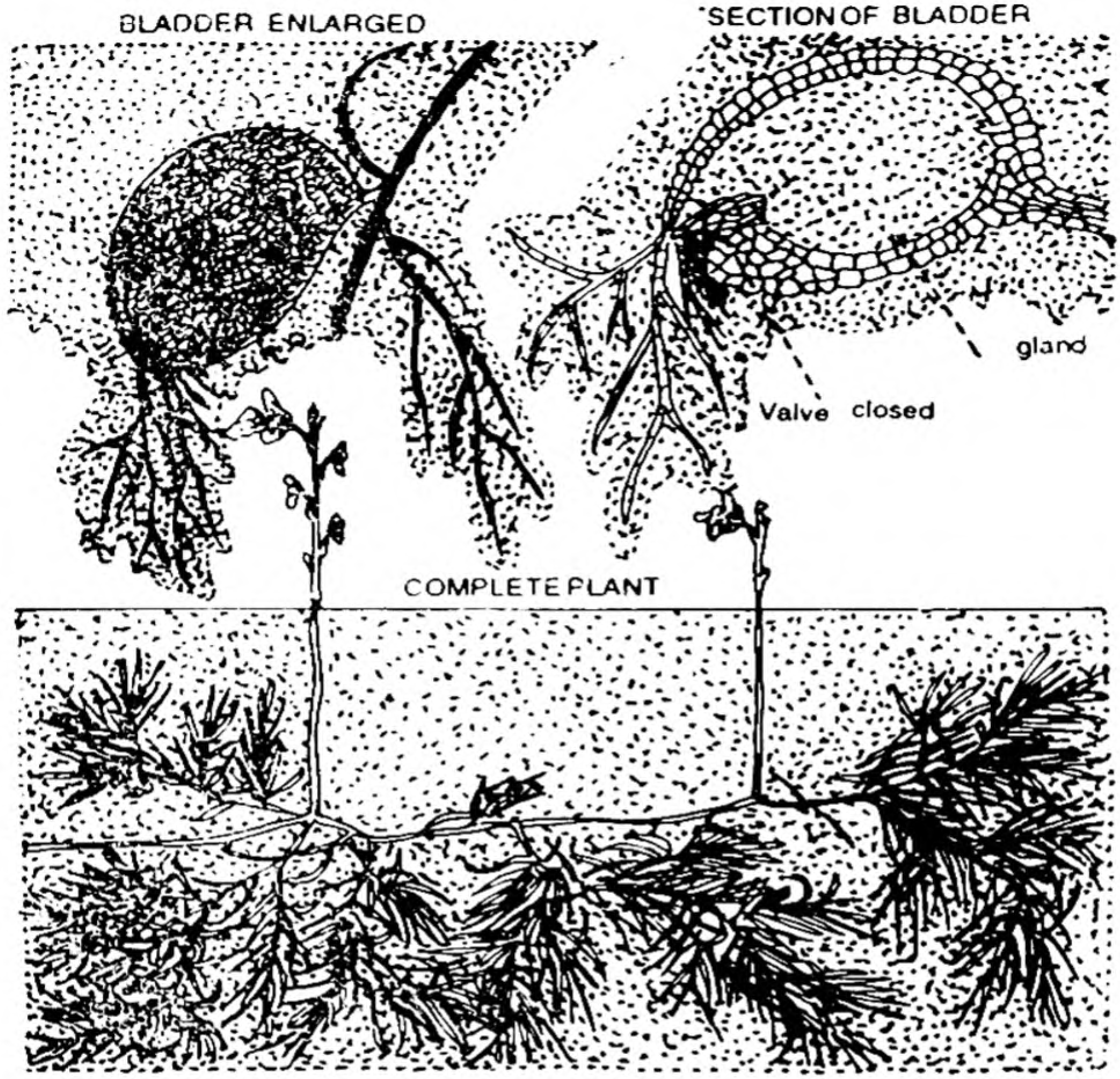
BLADDER ENLARGED
SECTION OF BLADDER
gland
Valve closed
COMPLETE PLANT

FIG. 6.3: Bladderwort.

enzymes are secreted by the bladder but the body of the prey is broken down by bacterial action. Some of the soluble nitrogenous substances so produced are absorbed by the wall of the bladder.

The trap is then set again, a process taking about twenty minutes. Curious four-rayed hairs clothe the inner surface of the bladder, and these absorb water (Fig. 6.1. E). Since the bladder, sealed with its valve, is impervious to water the volume of the bladder is decreased and the walls sucked in. A considerable tension is thus set up, and the bladder is again in the 'set' condition.

The flowers of this plant appear during June and July borne on long, thin stalks which project above the surface of the water. They are rich yellow in colour with a pronounced spur.

There is a smaller species of bladderwort known as the lesser or small bladderwort (*Utricularia minor*) which is more localised in distribution, mainly in the north. Its leaves have not so many forked lobes. Two other species are even more localised.

CARNIVOROUS PLANTS OF THE TROPICAL AND SUB-TROPICAL REGIONS

The carnivorous plants just described are the only ones which grow wild in Great Britain. This shows how comparatively rare carnivorous plants are. But there are a few very interesting plants which demonstrate this habit, native to tropical and sub-tropical regions.

One such plant is called *Venus's fly-trap* (*Dionaea muscipula*). This plant is very common in the peat bogs of North and South Carolina. The first full description of the insectivorous habit of this plant was given by Charles Darwin, though it had actually been noticed before. The insectivorous habit is again connected with the leaf. The leaves are arranged in rosette fashion, as in British terrestrial insectivorous plants.

Each leaf consists of a winged petiole which is joined by a short cylindrical region to the lamina, which is modified as a trap for insects. The lamina consists of two lobes united by a thick mid-rib. The margins of the lobes bear long, film spikes, while the surface of the lobes bears glands of two kinds. A narrow marginal zone is occupied by glands which secrete nectar, a sugary solution attractive to insects, while the rest of the surface bears digestive glands having a deep red colour. Each lobe also bears three stout bristles, each joined at the base and sensitive to touch.

When an insect alights on the surface of the lamina and moves about in search of nectar it cannot help but touch one more of the sensitive bristles. The result is that in less than a second the lobes snap together and the marginal spikes interlock with one another so that the lobes cannot be pushed apart. This first rapid movement effectively traps any large insect but allows the escape of small ones: it is followed by slower movements which bring the lobes closer together, and the lobes themselves become flatter so that the prey is held firmly between them.

The digestive glands secrete proteolytic enzymes and the products of digestion of the insect body are then absorbed. The lobes open again after some days.

The movement is 'triggered' by the bristles, which are able to fold over at their joints as the lobes close together. The closing movement is caused by a rapid increase in size of the lower leaf surface and the slow reopening by increased growth of the upper surface. Changes in turgidity and growth are both involved.

It is interesting to note the instructive experimental observation that Darwin made on this trapping mechanism. His experiment showed what a great deal of liquid containing the protein-digesting enzymes is secreted by the glands of this leaf. He waited until an insect was caught in a trap, then immediately made a small perforation at the base of the lobes, without removing the crushed insect. The digestive fluid poured out of this hole and flowed down the leaf-stalk, and it continued to do this for nine days.

Some carnivorous plants are known as *pitcher plants* since their leaves are modified to form receptacles or pitchers which act as passive traps for insects and other animals.

The genus *Nepenthes* is an outstanding example. It has about sixty species which are distributed throughout the old world tropics, extending from Madagascar to North Australia and being especially abundant in North Borneo.

The species are of varying size and habit; but the majority of them are plants of the wet jungle, growing rooted in the swampy soil or as epiphytes on the forest trees. They usually have a rhizome from which climbing shoots grow up through the jungle vegetation, sometimes to the tops of trees 50 ft. high. The leaves are highly modified both for climbing and as traps for insects. Each leaf has a basal region clasping the stem. Above this is a flattened region which resembles the lamina of a normal leaf both in appearance and function. Beyond this again is a cylindrical tendril of varying length bearing a pitcher at its tip. This pitcher is the last part to develop, and before it does so the tendril has often twined round a support, thus aiding the climbing habit and also serving to support the pitcher. The latter always assumes a vertical position in response to the stimulus of gravity, suitable growth taking place just below the pitcher. It has proved impossible to relate the various parts of this highly modified leaf to the parts of a normal leaf and many different interpretations have been advanced.

The pitches themselves are usually tubular, with the upper third somewhat constricted, and bear two wing-like structures down their length. The mouth of the pitcher, which has a conspicuous rim, is usually placed obliquely and is overhung by a lid. When the pitcher is young the lid tightly seals the opening; but, as the pitcher grows, it is raised and, remaining stationary, it serves to keep rain from entering the now open pitcher. The size of the pitchers varies in different species, from the size of a thimble to that of a quart mug. They are also variously coloured. The general colour is usually yellowish or bright green and there are blotches or stripes of bright colours, red, purple or yellow. Variations in the intensity of the colour are governed by factors such as light intensity and soil conditions. These bright colorations, like those of flowers, serve to attract insects.

The detailed structure of the pitchers is related to the attraction, capture and digestion of the prey. The lid, apart from keeping out rain. attracts insects by means of nectar secreted on its under surface. The pitcher itself has a conspicuous rim which forms a sort of parapet with a general tendency to point downwards into the pitcher. It is hard and glossy, with prominent ridges which end in sharply pointed teeth. Large nectar glands are present between the teeth. The inner surface of the pitcher is differentiated into two zones. In the upper zone, starting just below the rim, the epidermal cells secrete minute flakes of wax on their outer surface. The lower zone bears numerous glands, each being seated in a small depression, the upper rim of which overhangs the gland (Fig. 6.1, C and D). These glands secrete a slightly acid liquid during the development of the pitcher so that young pitchers, sealed with their lids, are already filled with a sterile fluid.

Insects are attracted to the pitchers by their bright colours and by the copious secretion of nectar by the glands on the under surface of the lid and on the rim. Small insects, such as ants, are able to walk on the glossy rim; but when they attempt to walk on the wall just below the rim they immediately slip down into the pitcher liquid. This is because the small flakes of wax produced there stick to their adhesive foot-pads and render them useless. Larger insects, such as cockroaches, beetles and flies, find the rim itself treacherous and, in attempting to reach the nectar glands on its inner margin, they too fall into the pitcher. These slipping zones are in fact extremely effective. The escape of any insect so trapped is prevented by the zone of wax and by the downwardly pointing teeth of the rim.

The insect soon dies in the pitcher liquid, possibly because the liquid contains a wetting agent. The presence of the insect results in active secretion by the digestive glands. This secretion is acid and contains an enzyme which breaks down the proteins of the insect body. The products of digestion are then absorbed by the glands. The enzyme is very active and insect bodies are disintegrated within forty-eight hours and only the chitinous skeletons remain.

A different type of pitcher is formed by the genus *Sarracenia* (side-saddle flowers). The nine or so species are all native to marshy habitats in the eastern regions of North America, extending from Labrador to Florida.

The rhizome bears a rosette of radical leaves. These have a broad sheathing base, above which the whole leaf forms a long, tubular pitcher. with a single photosynthetic wing down its length and a conspicuously coloured upright flap at its mouth.

The flap attracts insects by the secretion of nectar, and such insects tend to move towards the pitcher cavity since downwardly pointing hairs make movement in other directions difficult. The rim round the mouth of the pitcher secretes nectar. and nectar glands are also present on the inner wall below the rim. This upper zone of the wall is extremely slippery although no wax is present and insects fail to maintain their foothold. Below the slipping zone are numerous digestive glands. The amount of liquid secreted is small but contains enzymes similar to those af *Nepenthes*. Digestion and absorption follow as in the latter.

The cobra plant (*Chrysamphora*, formerly *Darlingtonia*) belongs to the same family as *Sarracenia*. The pitchers are bent sharply forward at the top and the small mouth faces downwards. The forked lid curves forwards and acts as a platform leading to the opening of the pitcher. The whole rosette of pitchers looks like "a number of hooded yellow snakes with heads erect, in the act of making a final spring." Insects landing on the flap are lured inside by nectar and fail to escape mainly because they are attracted to translucent areas, or windows, in the wall of the pitcher rather than to the actual opening which is no the shaded lower side. The trapped insects eventually die and, although no enzymes are secreted by the pitcher, their bodies are broken down by the action of bacteria and some of the simple nitrogenous substances produced are absorbed by the lower part of the pitcher wall.

The pitchers of the carnivorous plants just described are obviously efficient in the trapping and digestion of small animals. It is interesting to note, however, that a large variety of animals utilise the pitchers and their contents as homes and as a source of their own food supplies. For examples, certain species of mosquitoes and gnats breed exclusively in the pitchers of *Nepenthes*, and other species of the same insects only in those of *Sarracenia*. The larvae of these insects live in the fiuid of the pitchers. Protozoa, small crustaceans, diatoms and desmids also live in this fluid, and it is suggested that all these organisms are able to survive in a fluid which digests other animals by reason of their possessing substances which inhibit the action of the enzymes.

Pitchers frequently become filled with a decaying mass of insect debris. Flies of various species lay their eggs in this debris so that the developing larvae are provided with a plentiful food supply. Birds are attracted to the pitchers and feed on the larvae after slitting the pitcher walls with their beaks.

Other animals utilise the attractiveness of the trapping mechanisms. Thus, there are several species of spiders which are found living only in the mouths of the pitchers of *Nepenthes* and *Sarracenia*. Small toads and lizards may also live just inside the pitchers, and these, like the spiders, take their toll of the visiting insects.

It is clear, therefore, that a carnivorous plant is the centre of a whole living community, each member of which is adapted in relation to the very special conditions arising from the carnivorous habit.

7

Natural Cycles and Plant Life

The relationships of plants to soil and water, as described in the two preceding chapters, indicate how closely the organic and inorganic worlds are bound together. Plant life depends directly on about one sixth of the naeurally occurring chemical elements. The use of these elements and their compounds in metabolism and growth involves energy which came originally from the sun. In terms of the mass of the earth and the energy given off by the sun (in the temperature zone about 500 kcal. of solar energy strikes every square foot of land or water each day), the amount of matter and energy involved in the life processes of plants and animals is small indeed. But because plants are so abundant, the flow of materials and energy through them is on a vast scale. More important is the fact that much of this flow of matter and energy involves interactions between living and nonliving matter.

The organic-inorganic relationships of matter have been termed *cycles*. Perhaps the earliest recognition of a cycle appears in Genesis III: "Dust thou art, and unto dust shalt thou return." Numerous natural cycles are known: nitrogen, carbon, phosphorus, water, oxygen, and sulphur. Most of these involve changes in which the soil, plants, and animals large and small, all play important roles. Although each of these cycles may be isolated for identification and analysis, all are markedly interdependent, and operate continually and concurrently. The term *cycle* implies a return to a starting point, but this may often be a devious one in time and space. Although the broad outlines of the natural cycles are fairly clear, our understanding of the details of many of them is limited.

Some cycles involve only a physical change, as when liquid water is converted to water vapour and returns to the liquid state. The occurs during such processes as evaporation, transpiration, and condensation. Other cycles involve chemical as well as physical change. In all cycles energy is necessary to produce the physical and chemical changes and energy is either required or liberated as a result of them. An understanding of any cycle is not complete without a knowledge of the energy factors involved. Certain aspects of these energy exchanges will be considered later in this chapter.

THE WATER CYCLE

This most obvious of the natural cycles involves the movement of water from the earth and the seas into the atmosphere and back again. The supply of water of the earth is fixed and stable and is used over and over again. It is present chiefly as:

1. water vapour of the atmosphere, estimated at some 225 thousand billion tons;
2. water in the oceans, steams, and lakes;
3. water in the soil; and
4. water in living organisms.

The amount of water in each of these does not change appreciably.

We may begin our study of the water cycle (Fig. 7.1) by

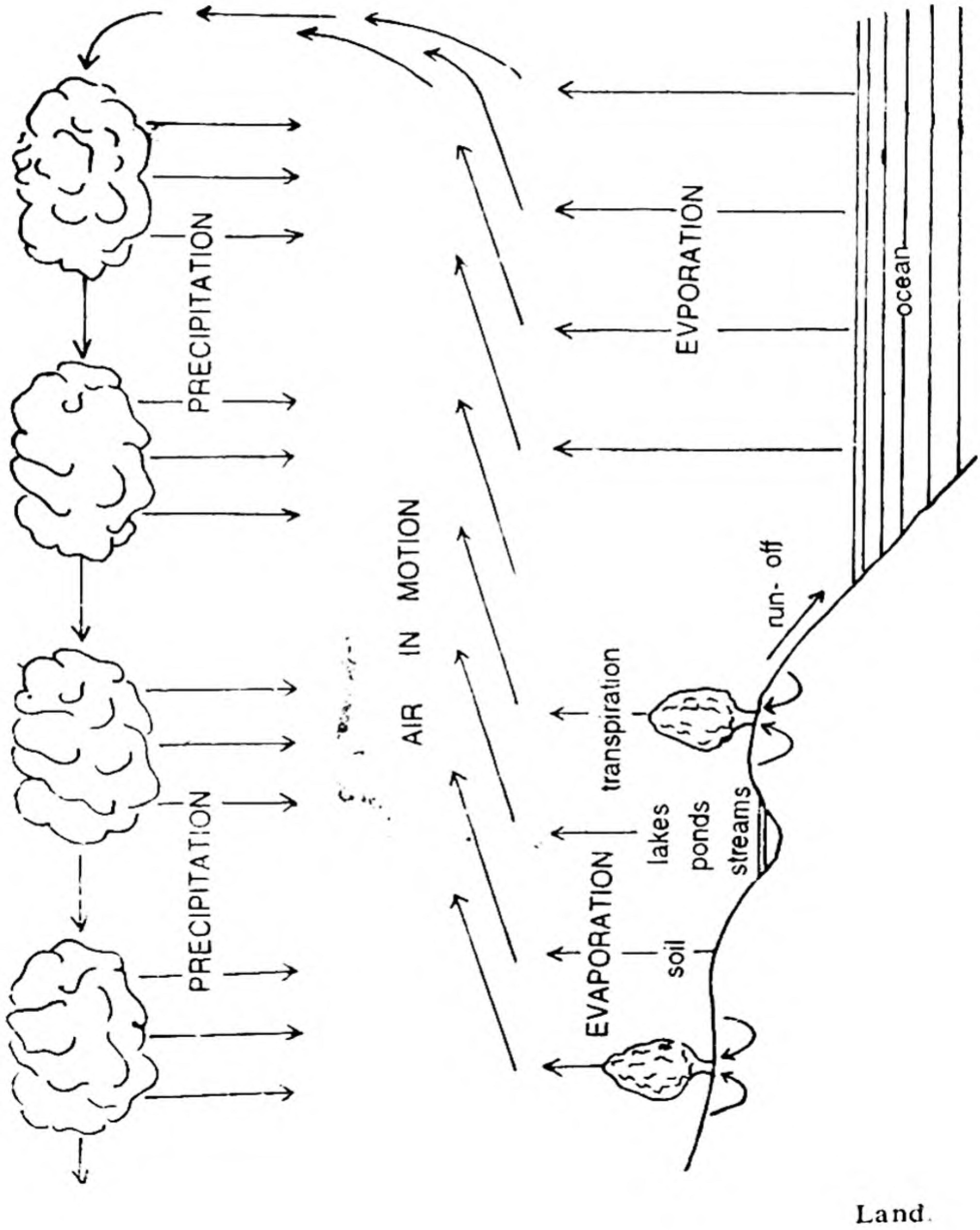
PRECIPITATION
PRECIPITATION
AIR IN MOTION
EVPORATION
ocean
run- off
transpiration
lakes
ponds
streams
EVAPORATION
soil
Land.

FIG. 7.1: The water cycle.

identifying the sources of atmospheric moisture. The radiant energy of sunlight, falling upon the earth's surface, brings about the daily evaporation of enormous amounts of water. This passes into the atmosphere from the oceans, lakes, and streams. It also comes from the soil surface, and from rain water evaporating from the surfaces of plants after a rainfall. A considerable part of the atmospheric water is water vapour released from plants in the process of transpiration. The same solar radiation which produces evaporation also causes the winds. Wind currents are important in determining the local distribution of atmospheric moisture.

Some of the land-evaporated moisture falls back upon the land, but much—perhaps most—of it is carried over the oceans. Here it becomes a part of the greater mass of water vapour derived from the ocean itself. Following circulation by winds, the atmospheric moisture is precipitated as rain or snow over the oceans and upon the land. The condensation of moisture during precipitation is accompanied by a release of energy in the form of heat, which is dissipated into the atmosphere.

This precipation upon the continents is one of the factors which make possible the existence of plants, animals, and man. Estimates available for the United States show that 10 to 50 per cent of the precipitation reaching the soil surface is returned to the oceans by run-off into streams and that 40 to 60 per cent penetrates into the soil. Much of this water ultimately also reaches the streams and rivers through discharge by springs and seepage. The water returning to the oceans brings with it chemical compounds derived from the soil, thus adding several billion tons of salts annually to the sea.

Much of the water which penetrates the soil is absorbed by roots and moves through the plants, returning to the air as transpired water vapour. It has been estimated that, in the United States, 15 to 30 per cent of the precipitation which percolates into the soil is taken up by plants. Most of this is transpired; a small part is used as one of the raw materials of photosynthesis, and some is used in other metabolic activities.

It is also the source of the oxygen which is released into the air from green plants. When a plant decays or is burned, water is released into the atmosphere. A definite but negligible amount of water also passes through the bodies of animals and is released during life or after death and decay.

The water cycle thus includes many subsidiary cycles and byways. Like photosynthesis, it is dependent upon solar energy. The water cycle is a relatively simple one, but it involves many phases of the organic and inorganic world. To it are related such essential human problems as soil erosion, water power, food supply, urbanization (water supply is a critical problem for many large cities), and flood control.

LIFE IN THE SOIL

As we indicated in the preceding chapter, soils consist not only of varying mixtures of sand, silt, and clay but also of nonliving organic materials and living organisms. Most soils are inhabited by vast numbers of bacteria and fungi. The bacteria alone may number hundreds of millions per gram of soil, depending upon the amount of organic material, the temperature, the acidity, and other factors. Fungi are especially abundant in acid forest soils of the temperate zones and in the acid soils of the humid tropics.

As a result of the activities of green plants, great amounts of essential elements are brought into organic combination. Since animals feed upon green plants, their bodies also contain these essential elements. The quantity of some of these elements, in forms which plant life can use, is limited. In the course of time they would become locked up in organic form and so become unavailable to future generations. But bacteria and fungi prevent this. They decompose the dead bodies of plants and animals and reduce them to water, carbon dioxide, ammonia, and simple mineral compounds. The essential elements are thus returned to the soil.

The reduction of the fresh organic materials to simpler end products proceeds gradually, and during the process many different compounds, known collectively as the soil *humus*, are formed. Humus serves as a reservoir of mineral nutrients which are gradually liberated in a form available to plants as the humus undergoes further decomposition. Most of the decay processes take place in the upper layers of the soil—the *topsoil* —which thus becomes rich in the residues resulting from decay. Thus the elements pass through a cycle in which they are a part successively of life, death, and decay. In the absence of this soil cycle, life on earth would eventually cease.

THE CARBON CYCLE

The carbon cycle, like the water cycle, is one of the simplest of the natural cycles. Yet it occurs on a scale that taxes the imagination. The amount of carbon dioxide in the air is relatively small—about 0.03 per cent by volume—but because of the enormous mass of the atmosphere this small fraction amounts to some 2200 billion tons, equivalent to about 600 billion tons of carbon. Carbon is continually being withdrawn from the air as carbon dioxide is used in photosynthesis. This withdrawal by land plants has been estimated at about 19 billion tons annually.

Some of the carbon so withdrawn is not returned directly to the atmosphere but is stored in various forms. It may, for example, be combined chemically to form calcium carbonate (limestone). This is brought about by the activities of many kinds of organisms, such as coral animals, shellfish, algae, and bacteria. Deposits of limestone are typically formed on the bottom of shallow seas. Deposits of coal and oil are natural reservoirs of carbon derived directly or indirectly from the carbon dioxide of the air. The carbon in these deposits is estimated at about 30,000 times that now present in the atmosphere. The storage of carbon still continues, as evidenced by the continues deposition of extensive beds of peat, oil shale, and limestone.

The amount of carbon dioxide in the atmosphere remains relatively unaltered, however, for other processes continually return it to the air. A tremendous amount of carbon dioxide—about 100 times as much as in the atmosphere—is dissolved in the waters of the oceans. This reservoir of carbon dioxide is believed to be a great stabilizing factor, maintaining the carbon dioxide concentration of the atmosphere at a constant level. Some carbon dioxide is returned to the air by the respiration of animals and green plants, and by the combustion of wood, coal, and oil. Contrary to popular belief, the total quantity of carbon dioxide released into the atmosphere by land plants is greater than that released by land animals. Considerable amounts of carbon dioxide are also supplied to the atmosphere as a result of volcanic activity.

In spite of these sources of renewal, the carbon dioxide supply of the air would eventually be depleted as a result of the utilization of this gas on an enormous scale in photosynthesis if the carbon removed from the air and built into the bodies of plants or of animals which feed upon plants were not made available again. When these bodies die and decay, they are acted upon by vast numbers of microscopic living things—bacteria and fungi. The respiration of these forms of life is probably the most important source from which carbon dioxide supply of the air is renewed. Since the amount of carbon dioxide in the atmosphere is limited, it is only because of the activities of organisms of decay that green plants can manufacture of food upon which the world depends.

The carbon of the earth's atmosphere is thus used over and over, passing through a natural cycle (Fig. 7.2). For unnumbered centuries, the carbon in our own bodies has circulated throughout the world in a vast cycle of eternal change—from the air to the green plant from plant to animal from animal to the bodies of organisms of decay, in one continual round. Any one of the atoms of carbon in the bread or meat which appears upon the table today may have formed a part of a plant or animal living millions of years ago, long before man, mammals, or flowering plants appeared upon the earth's surface.

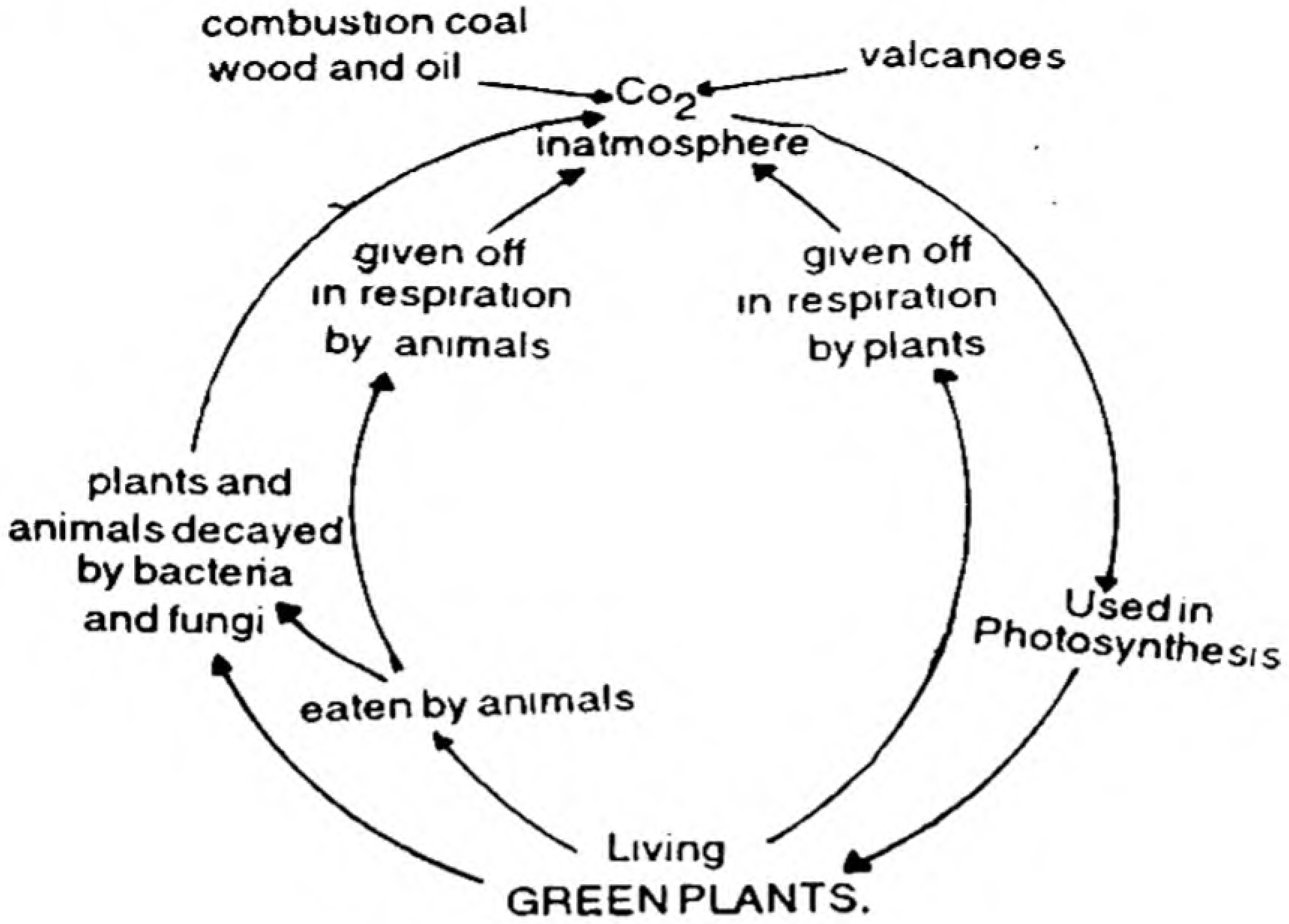
combustion coal
wood and oil
valcanoes
Co2
inatmosphere
given off
in respiration
by animals
given off
in respiration
by plants
plants and
animals decayed
by bacteria
and fungi
Used in
Photosynthesis
eaten by animals
Living
GREEN PLANTS.

FIG. 7.2: The carbon cycle.

THE NITROGEN CYCLE

The productivity of plants, both of the land and the sea, is largely dependent upon nitrogen. A deficiency of nitrogen is more commonly a limiting factor in plant growth than that of any other mineral element. This is indeed strange when it is realized that nitrogen constitutes about 80 per cent by volume of the earth's atmosphere. The air over a single acre of land contains about 35,000 tons of this element. But in an acre of soil the nitrogen amounts to only a few thousand pounds—rarely more than four tons. Unfortunately, the molecules of free atmospheric nitrogen are not available to green plants.

This dilemma of an abundance of an essential element which cannot be utilized directly was not generally recognized until the middle of the last century, when Boussingault, a French agricultural chemist, conclusively demonstrated, as a result of twenty years of experiments, that the nitrogen of the air could not be used by green plants.

In 1858, Boussingault planted three seeds of a small species of sunflower in separate pots, using pure sand instead of soil. The first plant was watered with distilled water. The second received a solution containing all the essential mineral elements except nitrogen. The third seed was given all the nutrient salts, including nitrogen in the form of potassium nitrate (KNO_3). After 86 days, only the sunflower which had been supplied with potassium nitrate showed normal growth, reaching a height of more than 2 feet. The other two plants, which had only the nitrogen present in the seeds, were only about one seventh as high. The plants were removed, dried and analyzed. Here are the results:

Plant	*Gain in dry weight, mg.*	*Gain in nitrogen, mg.*
Sunflower on sand and water	0.28	2.3
Sunflower on sand with mineral salts except nitrogen	0.39	2.7
Sunflower on sand with complete nutrient medium	21,111	116.7

Boussingault attributed the gain in nitrogen by the first two plants to the presence of ammonia in the air. Later experiments, with improved techniques, have confirmed the fact that uncombined nitrogen, the form in which the element is chiefly found in the air, cannot be utilized directly by plants.

The organic matter of the soil consists largely of plant remains. Animal wastes and the remains of dead animals also contribute to the organic matter, which contains nitrogen in the from of proteins or related compounds. This nitrogen, for the most part, is no more available to green plants than is the uncombined nitrogen of the atmosphere. The nitrogen supply of plants is derived from the soil, but chiefly in an inorganic form in which the nitrogen is chemically combined with the elements hydrogen or oxygen. Such chemically combined nitrogen is produced during the decomposition of organic matter by the bacteria and fungi of the soil.

Ammonification. As a part of the process of decay, bacteria convert the protein materials of dead bodies and organic fertilizers into ammonia (NH_3), a compound of nitrogen and hydrogen. This occurs at the end of a long series of digestive activities, during which the proteins are broken down by enzymes into amino acids and these in turn into ammonia. carbon dioxide, and other products. The decay organisms obtain energy and food materials for their own bodies during the later stages of the process.The ammonia thus formed dissolves in the soil water.

Many species, perhaps the majority of bacteria living in the soil, are able to transform the nitrogen of proteins into ammonia. Such organisms are called *ammonifying* bacteria, and the process is known as *ammonification*. Ammonification is also brought about by fungi, especially in acid soils not favourable to bacterial growth. The ammonia may be absorbed directly by green plants and used in the synthesis of proteins or it may be acted upon by other kinds of bacteria to form nitrates (NO_3). compounds containing nitrogen and oxygen.

Nitrification. Nitrogen is also available to plants in the form of nitrates produced by the action of two kinds of bacteria upon ammonia. The conversion of ammonia to nitrate is known as *nitrification*, and the bacteria are known as the *nitrifying* bacteria. Nitrification takes place at or near the soil surface. Although only a few species of bacteria are involved, the individual organisms are numerous and widely distributed in cultivated and uncultivated soils.

Nitrification takes place in two steps and involves two kinds of nitrifying bacteria, one kind of organism acting upon the product of other. In the first step the ammonia is taken into the bacterial cell and there oxidized to *nitrite* (NO_2). Nitrites do not accumulate in the soil, for the second step follows quickly—the nitrites are oxidized to nitrates by the second group of nitrifying bacteria. The energy released in both of these processes is the only energy available to the nitrifying bacteria. It is used to construct cell walls and build protoplasm and to synthesize food from the carbon dioxide and water of the soil.

The nitrifying bacteria, then, are generally similar to green plants in that they, too, are autotrophic. Both can manufacture food from simple raw materials. The energy used by green plants in food manufacture is the radiant energy of the sun, and the process takes place in the light. The nitrifying bacteria, however, employ chemical energy obtained by the oxidation of inorganic compounds, and are able to synthesize their food in the dark. This method of obtaining food is termed *chemosynthesis* as distinct from photosynthesis. The nitrifying bacteria are readily affected by adverse environmental conditions. Nitrification proceeds most vigorously if the soil is well aerated, at temperatures of about 27°C. (80°F.), and if lime and other essential elements are available in abundance.

The production of nitritates from organic matter in the soil was once regarded as a purely chemical process. That it is biological and not chemical was discovered by two French investigators, Scholoessing and Muntz, in 1877. Stimulated by the work of Pasteur, these workers were trying to discover a method by which the discharges from the severs of Paris could be made harmless and so could be used to irrigate fields near Paris. They passed diluted sewage through long glass tubes filled with soil. They found, after 20 days, that the sewage lost ammonia but gained in nitrate. The nitrifying process was stopped when chloroform vapour was forced through the tubes. Nitrates were again formed, however, if an infusion from fresh garden soil was added to the tudes. Finally, the process stopped if the tubes were heated to 100°C. The result of these experiments indicated that bacterial action was responsible for nitrification, and this conclusion was soon confirmed by other workers. It was not until 1890, however, that the bacteria concerned were isolated and identified.

The belief was once current that green plants could use only nitrates as a source of nitrogen. It has now been established that they can utilize the nitrogen from ammonia as well as nitrates. In many species, normal plant growth may occur is the presence of ammonia nitrogen alone. Most investigators consider, however, that nitrates are the most important and most available form of nitrogen for cultivated crops under the usual field conditions.

Nitrogen lost to plant life. Ammonification and nitrification do not increase the total amount of nitrogen in the soil. They merely convert the organic nitrogen in the tissues of dead plants and animals into a form which plants can use. The fundamental and most significant part of the nitrogen cycle is therefore the movement of nitrogen from the soil into the plant and from plant or animal tissues back again into the soil (Fig. 7.3).

There are, however, many leaks in the cycle. Nitrogen is continuously being lost from the soil. In cultivated soils

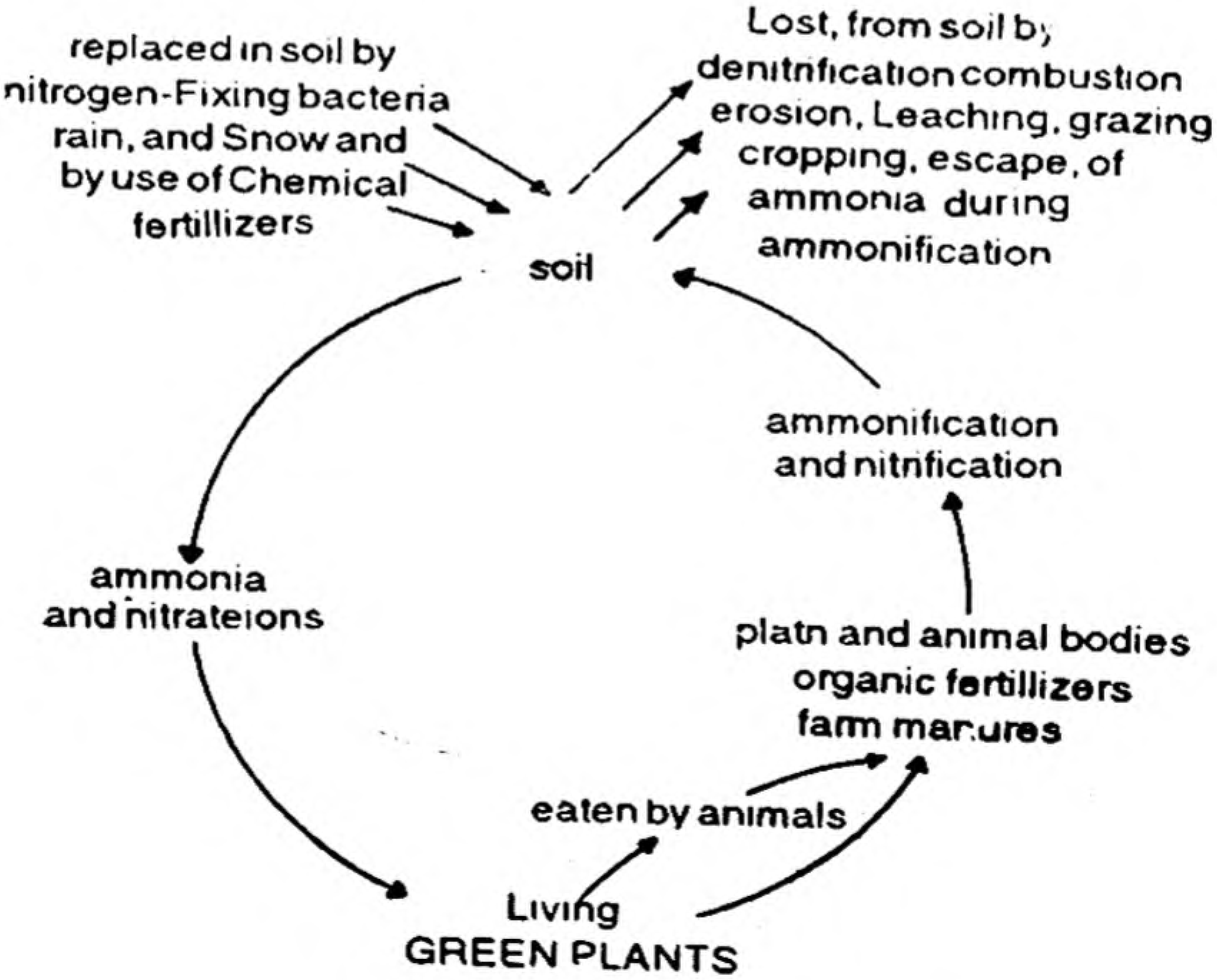
replaced in soil by
nitrogen-Fixing bacteria
rain, and Snow and
by use of Chemical
fertillizers
Lost, from soil by
denitrification combustion
erosion, Leaching, grazing
cropping, escape, of
ammonia during
ammonification
soil
ammonification
and nitrification
ammonia
and nitrateions
platn and animal bodies
organic fertillizers
farm manures
eaten by animals
Living
GREEN PLANTS

FIG. 7.3: The nitrogen cycle.

the chief causes of this loss are leaching, crop removal, grazing, and erosion. The loss of nitrogen, principally in the form of nitrates, by erosion and leaching is great, since nitrates are highly soluble. Plants or parts of plants are removed by grazing or harvesting. In the harvest of an acre of alfalfa, 190 pounds of nitrogen are removed from the soil. An acre of corn takes 110 or more pounds of nitrogen; wheat 51 pounds, and potatoes 63 pounds.

The nitrogen supply is not static. It is constantly being used, depleted, lost, and restored. The annual loss of nitrogen from the cultivated soils of the United States, through leaching, cropping, grazing, and erosion is estimated at nearly 23 million tons. This loss is in part replaced to the extent of about 16 million tons, resulting in an annual net loss of more than 6 million tons. The losses of other elements are even greater; the net annual loss of potassium from the soil is about 45 million tons, and of calcium more than 55 million tons.

Other smaller but still significant nitrogen losses occur. The burning of forests, grasses, crop residues, and other organic matter releases large amounts of nitrogen into the air as oxides of nitrogen. During the process of ammonification, some nitrogen in the form of ammonia is lost from the soil into the air. And a large but undetermined amount of nitrogen is lost from the soil through the action of *dentrifying* bacteria. These bacteria are anaerobic, obtaining their energy by the reduction of nitrates and ammonium compounds, thus releasing ammonia or nitrogen in the elemental form. The dentrifyers occur chiefly in wet, uncultivated soils.

The nitrogen content of soils varies considerably. In the rich prairie and chernozem (dark-coloured) soils, the amount of nitrogen has been estimated at 8000 pounds an acre to a depth of 40 inches. There are about 123 million acres of thesesoils through the Middle West. Lowest in nitrogen are the 150 million acres of red and yellow soils of the Southest, which average only about 2000 pounds per acre.

Nitrogen restored to the soil. It is evident that our soils are declining in fertility and that every effort must be made to replace and prevent the losses of essential elements. The nitrogen losses are in part balanced by:

1. the application of nitrogen in the form of commercial fertilizers, crop residues, green manure, and farm manure,
2. nitrogen brought down in solution in rain and snow. and
3. the activities of nitrogen-fixing bacteria.

More than 18 million tons of commercial fertilizer, with an average nitrogen contenr of about 5 per cent, are used in the United States each year. A small amount of nitrogen, perhaps 5 pounds per acre, is annually washed out of the atmosphere and added to the soil. This nitrogen exists in the air chiefly in the form of ammonia, much of it of organic origin, liberated during the decay of organic materials. The outstanding and perhaps the most important way by which the nitrogen of the air enters the soil, however, is by the activities of nitrogen-fixing bacteria.

Symbiotic Nitrogen Fixation

Three hundred years before Christ, Theophrastus wrote that the Greeks used crops of broad beans to enrich the soil. The Romans, according to Pliny, raised similar crops and turned them under as green manure. They recognized the value of lupines and alfalfa in the enrichment of the soil.

The crops so valuable in this way are the legumes, a large and important family of flowering plants which include the clovers, alfalfa, soybean, peanut, vetch, pea, bean. and many others. The value of these plants is due to the activities of specialized bacteria which live in swellings, called *nodules*, or *tubercles*, on the roots. The bacteria penetrate the root hairs and, having gained entrance, stimulate the cells of the cortex to divide, possibly through the action of growth hormones produced by the leaves of legume. The dividing cortex cells produce the nodules in which the bacteria live.

The legume bacteria are able to convert the uncombined nitrogen of the air into an organic form. No higher green plants can do this. The bacteria combine nitrogen from the air of the soil with carbyhydrates produced by the legume to form proteins. The sugar formed by the lugume is used not only as a component of the protein but also as a source of energy for this process, which is known as *n trogen fixation*, However, the mechanism of the process and the stages by which it proceeds are largely unknown.

The protein so manufactured from carbohydrates and nitrogen of the soil air is used by the bacteria in growth, but the legumes also profit. The nitrogen compounds are made in excess and are yielded to the plant by the bacteria, possibly in the form of amino acids, although investigators are not in agreement on this point. Both the bacteria and the legume thus benefit by this relationship. a condition called *symbiosis*, or the living together of two or more organisms in an association which is mutually advantageous.

Nitrogen fixation proceeds in nature only when the two organisms are combined; it cannot be carried on either by the bacteria or the legume alone. If the legume possesses no tubercles, its entire nitrogen supply must come from the soil. Even if nodules are present, some of the nitrogen supply is obtained from the soil.

The work of many men was involved in the series of discoveries which led to an understanding of nitrogen fixation by legumes. Boussingault and others had established that green plants could not use atmospheric nitrogen. However, it gradually became recognized that the legumes differed fundamentally from other green plants with respect to their source of nitrogen, Experiments showed that the legumes in some way could acquire more nitrogen than was present in the soil, the seed, or water. Finally, in 1888, two, German botanists, Hellriegel and Wilfarth, reported the results of extensive experiments in which they showed that legumes obtain nitrogen from the air through the activities of microorganisms which live in nodules on the roots.

In one of these experiments they grew peas and lupines in sterile sand. To some pots they added an extract of fertile soil in which these legumes had previously been grown. In such pots the legumes produced nodules, grew well and gained in nitrogen. This extract, of course, contained bacteria of the proper kind. Peas and lupines in sand to which the extract was not added grew poorly and soon died. The same experiment was carried out with oats, but the addition of the soil extract produced no results. That the effect of the soil extract was not due to the combined nitrogen it contained was shown by heating it to a temperature of 70°C. (158°F.), which killed the bateria. Plants to which such a heated extract was added behaved in the same way as those in the sterile sand alone. Thus the significance of the nodules, whose presence had long been known, was explained.

Legumes are important because of their own values and for improving the yields of other crops. Nonleguminous plants grown in combination with leguminous plants (timothy grown with clover or alfalfa, for example) benefit from the association. It is probable that the nitrogen-rich roots and tubercles slough off and decay in the soil, eventually releasing nitrogen in inorganic compounds. The stubble and roots of many perennial legumes left behind after the plants are harvested for hay are sufficiently rich in nitrogen, to aid in maintaining soil fertility and to increase the yield of following crops.

The greatest benefit to the soil and to succeeding crops results, however, from plowing under a leguminous crops as *green manure.* This increases not only the nitrogen content of the soil but also the organic matter. The organic nitrogen, following ammonification and nitrification, becomes, available to green plants. The stimulating effect of green manure upon crop growth is usually apparent for several years.

The amount of nitrogen fixed annually by symbiotic bacteria varies with the nature of the soil, the species of legume, and other factors. A few nodule organisms have been to fix as much as 250 pounds of nitrogen per acre annually, but the average is perphas 80 pounds per acre. It is estimated that some 5½ million tons of symbiotically fixed nitrogen are added annually to the soils of the United States.

Symbiotic nitrogen fixation will proceed only when certain bacteria are present. Hence it is usually advantageous to introduce the bacteria when the leguminous crops are planted, for they may not be present in the soil. The nodule bacteria are added to the seeds at the time of planting. The bacteria are first grown in large numbers in a nutrient medium, or food supply, under conditions that exclude other bacteria or molds. Such a growth is termed a pure culture. Cultures of nitrogen-fixing bacteria, combined with some kind of carrier, are sold under various trade names by commercial seed firms. Mixture of the bacteria with the seed just before planting ensures their presence at or near the roots at the time of germination. Research has disclosed various strains or varieties of the nitrogen-fixing bacteria. A given strain will infect not all kinds of legumes but only those within particular groups. These groups which require different varieties of bacteria are seven in number: alfalfa, cover, pea bean, lupine, soybean, and cowpea. The addition of lime to the soil favors bacterial growth, by neutralizing soil acidity. Nodule formation is best at or near neutrality.

Free-living nitrogen-fixing bacteria. Bacteria living on organic matter in the soil, as well as symbiotic bacteria, are able to fix atmospheric nitrogen. Some of these nonsymbiotic nitrogen fixers ate aerobic and some are anaerobic. All use energy obtained, from organic materials to combine carbohydrates and elemental nitrogen to form protein. The growth and death of these organisms increases the nitrogen content of the soil.

Nitrogen fixation occurs only in neutral or slightly alkaline soils and is stimulated by the presence of organic materials rich in carbohydrates. Estimates of the amount of nitrogen fixed annually by these bacteria range from 10 to 40 pounds per acre. It is not possible at present to increase the numbers of nonsymbiotic nitrogen-fixing bacteria in the soil by artificial inoculation.

ENERGY TRANSFORMATIONS AND NATURAL CYCLES

The Source of Energy

Tranformations of energy, unlike those of matter, do not occur in cycles. Nearly all the energy of the earth is derived directly from the sun. This radiant energy is transformed into many other kinds of energy, but it is finally lost to the earth in the form of heat, radiated out into space. Energy has alread been defined as the capacity to do work. The transformation of radiant energy into chemical, mechaeical, electrical, and other forms of energy involves many natural and industrial processes. In the movement of energy to, upon, and from the earth, all natural and human activities find their place. All the cycles so far described may be considered as illustrating some of the devious paths in the flow of energy.

Some energy is released by the disintegration of radioactive elements in the crust of the earth, but this amount is small compared to the radiant energy of the sun, our chief source of energy. The radiant energy reaching the earth's atmosphere is estimated at 125×10^{21} kcal. per year. About 60 percent of this energy is reflected or absorbed by the atmosphere and clouds, so that the amount of energy reaching the surface of the earth is considerably reduced. But only a part of this remaining energy is directly available to living things. Much of it warms the rocks and the soil, or is absorbed by or reflected from the seas. This portion of the solar energy is important in bringing about the evaporation which sets the water cycle in motion. Water is made available to land plans in this way and we benefit indirectly by obtaing water power for hydroelectric and other purposes. The energy used in the evaporation of water, as we pointed out previously, is released when water vapor condenses to form rain or snow.

Energy and Life

Of the total amount of solar energy with reaches the earth, some 3×10^{13} (3 quintillion) kcal is used annually in photosynthesis. The efficiency of photosynthesis is low, but the result of the process is the annual production of approximately 300 billion tons of sugar. As soon as photosynthesis is completed, the stored chemical energy begins to be dispersed in many ways. From the time radiant energy enters the body of green plant it moves in a constant downhill flow which ultimately leads to its dissipation into space. According to the low of conservation of energy, energy cannot be destroyed; it can, however, be lost as far as living systems are concerned.

The energy relationships of the carbon cycle are intimately related to the fate of the chemical energy of the carbon compounds of the plant. The energy is released during respiration. Some is used in metabolic processes, and some escapes as heat energy into the air. When the plant dies and falls upon the soil, it is attacked by a multitude of soil organisms which utilize the stored energy, but in so doing they transmit a portion of it into the atmosphere in the form of heat. If the plant is consumed by an animal, as when a herbivore eats grass, the stored energy of the plant is transmitted to the animal. In the body of the animal, energy is set free, much of it in the form of heat. The animal body, in turn, finally decays, with a release of heat energy. Energy in the form of heat is also dissipated into the air in the process of nitrification —only a small part of the energy resulting from the oxidation of ammonia and nitrite is utilized in the synthesis of organic matter. And so, whenever carbon dioxide and water are combined to form sugar and oxygen, it is new energy that is stored; while carbon and other elements circulate and are used repeatedly, energy is constantly lost as waste heat from plant or animal bodies and cannot be recaptured in the cycles of matter.

For thousands of the years man was dependent upon the current output of solar energy. He utilized this energy from his daily food, from the wood which he burned, from the labour of his domesticated animals, and to a limited extent from water power A few centuries ago, however, man began to utilize sources other than contemporary energy. This at first was coal. later supplemented by other kinds of foil energy—oil and natural gas. These sources of enery made possible the steam engine, the locomotive, steam-generated electrical power, the intermal-combustion engine, the automobile. the airplane, and diesel-generated electric power.

These sources of energy further emphasize the role of plants as energy transformers. Bituminous coal supplies about 48 per cent of our energy, anthracite about 6 per cent, Of the other principal fuels of organic origin, petroleum provides about 32 per cent of our energy and natural gas about 10 per cent. Less than 4 percent of the total power we use comes from water power. About 65 per cent of the electrical energy used is obtained from generators operating on organic fuels, and only about 35 per cent from hydroelectric sources. It is apparent that the power supply that makes modern civilization possible is still obtained in large measure from solar energy through the activities of green plants that lived millions of years ago.

SOIL AND EROSION

Erosion is the wearing away of the land surface by natural agencies, chiefly water and wind. Soil erosion is of two types.

1. Natural, or normal, erosion occurs in a natural environment, undisturbed by man. The presence of the native vegetation, with its ground cover of plant remains. retards the movement of soil to a peace no more rapid than that at which new soil is formed by weathering

processes and by the accumulation of organic matter. At most, natural erosson may remove a few inches of soil per country. Soils covered by native vegetation retain their fertility, for the soil constantly receives a supply of organic matter. There is no removal of vegetation; the entire plant, woody or herbaceous, sooner or later falls to the ground and decays, and the constituent elements return to the soil.

2. Accelerated erosion follows changes in the natural vegetation brought about by the activities of man. When man occupies the land, he cultivates and removes the products of the harvest, and his animals destory the grass. The soil is denuded, declines in fertility, and is gradually washed or blown away unless it is protected and used properly.

The early settlers in this country found in the East dense forests. clear streams. and rich soil. and in the Middle West mile after mile of grasslands covered with tall native grasses. There was little or no accelerated erosion. But as settlement and agriculture moved westward, accelerated erosion followed the ax and the plow. The covering of trees and gasses was removed and the soil was exposed to the destructive effects of wind and rain. Continual planting of soil-depleting crops robbed the topsoil of its fertility. In many areas unprotected soil was carried away by water erosion, thinning the slopes and hillsides and increasing the soil deposits in the flood plains of river valleys.

The total land surface of the United States, exclusive of mountains and deserts, is approximately 1758 million acres. The United States Soil Conservation Service estimates that 282 million acres have been either ruined or severely damaged by soil erosion. Fifty million acres of farmland have been abandoned: an additional 755 million acres have lost from one fourth to three fourths of their topsoil. Wind and water erosion remove about 3 billion tons of soil from the country's croplands and pastures every year. Accelerated erosion has become an economic and a social menace.

The better use of the soil and the proper management of the land are complex subjects which can barely be touched upon here. They represnt broblems which in part must be solved by engineering skills but which also involve a fundamental understanding of plants and the soil, especially from the standpoint of the requirements of the plant fot water, oxygen, and essential mineral elements. They are problems of primary concern not only to dwellers upon the land but also to those who live in cities. Society as a whole, as well as the farmer, is affected by abandoned farms, destructive floods, dust storms, eroded hillsides. and reduced crop yields.

Soil Conservation

The conservation of the soil and the maintenance of soil fertility are intimately related, Most procedures in land management are useful in the accomplishment of both objectives. An outstanding aspect of the preservation of soil fertility is the regular return of organic matter to the soil. This may be accomplished by the addition of farm manures and crop residues and by the growing and plowing under of grasses and legumes.

Organic matter serves as a reservoir of plant nutrients. It also aids in the prevention of erosion, for soils rich in humus absorb water readily and the of run-off is much less than from soils low in organic content. The addition of organic matter also increases the air capacity of the soil and stimulates the activities of microorganisms.

Soil depletion is accelerated by overgrazing and by the repeated growing of the single crop, such as cotton, corn, or tobacco, When the soil on farms becomes low in organic matter and essential minerals, a vicious cycle of depletion and erosion is set up. As the soil is depleted, the plant cover becomes poorer and poorer and the soil is more exposed to water and wind erosion. Erosion removes the topsoil, and the destructive forces are accelerated.

Soils from which the essential elements have been depleted can be restored by the addition of organic matter and commercial fertilizers. But the restoration of an inch of topsoil removed by erosion may require many years. The loss of nutrients not only results in lower crop yields but also may affect the health of animals which feed upon plants grown on exhausted soils. Animals require most of the elements essential to plants, together with sodium, chlorine, cobalt, iodine and perhaps others. Bone diseases of cattle are recognnized in regions lu which the soil is deficient in calcium and phosphorus, and other deficiency diseases result from an inadequate supply of iron, cobalt, manganese, copper, and iodine. The effect of soil nutrient deficiencies upon human health is a subject of vast importance which is being studied extensively at present.

Methods of soil conservation. Methods for the conservation of soil and water are applied according to the requirements of the area involved. Among these methods are the replacement of a cover of vegetation by replanting forests or reseeding grassland. plowing and strip cropping, cropping, terracing, control of grazing, and rotation of crops are widely practiced. The use of these and other conservation measures is encouraged by local, state, and federal agencies.

Trees, grass, and other close-growing vegetation are among the most effective agents in the control of erosion. In the forest, the blanket of leaf littler and humus is the primary agency holding the soil—not the tree root, as is sometimes supposed. Such a blanket breaks the impact of rain upon the soil surface. It absorbs rain water and melted snow, thus helping to prevent quick run-off and disastrous floods. The fibrous roots of perennial grasses and legumes bind the soil particles together, and the tops of the plants protect the soil surface from beating rains.

Contour strip cropping is practice of growing farm crops in long strips, across the line of slope, following the contour of the land. Strips of dene-growing crops, such as cereals, alfala, or grasses, are alternated with such clean-tilled crops as cotton, corn, or potatoes. This arrangement checks the momentum of run-off, filters out the soil carried from the tilled areas, and increases the absorption of rain water.

Crop rotation is the time-honored method of planting crops in a sequence on the same area over a period of years. The rotation may be a 2-, 4- or 5- years cycle including close-growing soil-improving crops such as grasses and legumes, and clean-tilled or soil-depleting crops such as corn, cotton, tobacco, or potatoes. An example of a crop rotation is the growing of corn, oats, and wheat one year, followed by two years of clover and grass.

Legumes are important components of a rotation. They may be plowed under as green manure of harvested for hay. Crop rotations, especially when used with farm manures, lime, and commercial fertilizers, are valuable in the control of erosion, weeds, and many plant diseases.

The soil supports man and all other animals and all plants that live upon the land. Without, soil, the land areas of our earth would be barren wastes. The conservation of the soil, the establishment of a stable, scientific, agriculture, and the efficient use of the land are important national problems. The results of unchecked soil erosion are evident over great areas of Africa, China, the Middle East, the Mediterranean region, India, and Latin America. In many parts of the world rich farmlands have been replaced by deserts of rock and shifting sand. Uncontrolled soil erosion can lead to national extinction just as certainly as the more rapid and dramaticdestruction of modern warfare.